UTILIZATION OF APPLE WASH TREATMENTS AND ULTRAVIOLET LIGHT FOR THE ELIMINATION OF *ESCHERICHIA COLI* O157:H7 IN APPLE CIDER

by

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ABSTRACT

Three studies regarding *Escherichia coli* O157:H7 in apple cider were conducted. The objectives were: to evaluate the effectiveness of wash and sanitizers for removing *E. coli* O157:H7 from apples; to survey cider producer practices; and to determine the efficacy of ultraviolet light for reducing *E. coli* O157:H7 in cider.

Apples with a five-strain acid resistant mixture of *E. coli* O157:H7 were treated with 200 ppm hypochlorite, a phosphoric acid-based fruit wash, 5% acetic acid, 5% acetic acid followed by 3% hydrogen peroxide, a peroxyacetic acid-based solution, and distilled water. The water wash caused insignificant reductions. All other treatments caused significant reductions. Acetic acid and peroxyacetic acid were the most effective with reductions of 3.1 and 2.6 logs, respectively.

The survey determined that most producers are small, seasonal operations. Most use sound orchard management practices, clean and sanitize daily, sort and wash apples,

use refrigeration, and try to prevent contamination. However, some use drop and damaged apples. Few use chemical sanitizers on apples, preservatives, pasteurize cider, or have HACCP programs.

Cider inoculated with the same mixture of *E. coli* O157:H7 was processed using a thin-film ultraviolet disinfection unit operating at 254 nm. Dosages ranged from 9,402 to 61,005 µW-sec/cm^2. Treatment significantly reduced *E. coli* O157:H7 ($p \leq 0.0001$) with a mean reduction of 3.81 log CFU/ml. Reduction was also affected by the level of background microflora in cider. Results indicate that ultraviolet light can reduce this pathogen in cider. However, additional reduction measures are necessary to achieve the required 5 log reduction.

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INTRODUCTION

The 1997 apple crop in the United States was valued at over 1.5 billion dollars, giving some indication of the importance of apples as an agricultural product (U.S. Apple Association, 1999). Approximately 21% of the 1997 apple crop was processed into juices including apple cider (U.S. Apple Association, 1999). In the U.S., the term apple cider is used to describe the unfermented, unclarified, and oxidized juice of freshly pressed apples, which is the form of juice most commonly consumed prior to 1940 (Downing, 1989). Today, pasteurized apple juices that are canned and bottled predominate (Downing, 1989). However, apple cider has its own distinctive appeal, is desired by many consumers, and provides an important source of income for apple growers (USDA, 1977).

Cider producers have typically relied upon its acidity as well as refrigeration, and chemical preservatives for preservation (Downing, 1989). Until recently, producers of unpasteurized cider were primarily concerned with prevention of fermentation and spoilage by yeasts, molds, and bacteria (Doores, 1983). However, an emerging pathogen has increasingly been associated with unpasteurized apple cider and juices.

The pathogen, *Escherichia coli* O157:H7, which had been primarily associated with beef (Borczyk et al., 1987; Griffen and Tauxe, 1991), has been identified as the agent responsible for outbreaks of illness associated with the consumption of unpasteurized juice and cider (Besser et al., 1993; CDC, 1996; CDC, 1997). Illness due

to *E. coli* O157:H7 is usually severe and is expressed as one of three syndromes, including hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP) (Padhye and Doyle, 1992).

These outbreaks have generated much concern and have incited research into the survival and growth of *E. coli* O157:H7 in acidic food products such as apple cider. Unlike many pathogens, *E. coli* O157:H7 has the unique ability to survive under acidic conditions (Doyle et al., 1997). In addition, survival at low pH is often enhanced at temperatures used for refrigeration (Miller and Kaspar, 1994). Survival in apple cider stored at 4^{o} C for extended periods has been documented (Miller and Kaspar, 1994; Zhao et al., 1993). Despite its ability to survive under such stressful conditions, *E. coli* O157:H7 is not particularly resistant to heat and is easily killed by pasteurization (Doyle and Schoeni, 1984).

The seriousness nature of the syndromes caused by *E. coli* O157:H7 and the results of research on its survival characteristics have prompted concern by consumers, producers, and government agencies alike. Many believe that pasteurization is the best means of eliminating *E. coli* O157:H7 from apple cider. While the FDA has not yet called for mandatory pasteurization of unpasteurized juices, it has proposed a regulation requiring processors to implement HACCP programs. This regulation would also require processors to adapt processes to achieve a five log reduction in pathogens in the finished product, the same level of reduction achievable through pasteurization (FDA, 1997).

Many larger processors have begun using a pasteurization procedure (Aylsworth,

1997). However, pasteurization is an unpopular option among most producers due to the costs involved (Kozempel et al., 1998). Pasteurization is not economically practical for most smaller, seasonal operations (McLellan and Splittstoesser, 1996). There is also concern about the effects of pasteurization on the sensory characteristics responsible for the appeal of fresh cider (Parish, 1997).

Therefore, researchers have been seeking viable and possibly, more economical, alternatives to pasteurization to help assure the safety of apple cider while preserving its sensory appeal. The overall objective of this study was to evaluate the effectiveness of two such alternatives. We investigated the effectiveness of chemical sanitizers for the removal of *E. coli* O157:H7 from the surfaces of apples as well as the efficacy of ultraviolet light for the reduction of this pathogen in apple cider. In addition, A survey of Virginia cider producers practices was conducted to gain a better understanding of current production practices.

SECTION I: REVIEW OF LITERATURE

A. *Escherichia coli* O157:H7

1. Characteristics

Escherichia coli is a gram negative, facultative anaerobic, nonsporeforming rod in the family Enterobacteriaceae (Holt et al., 1994). *E. coli* is considered a common inhabitant of the intestines of humans and animals and consequently is regarded as an indicator of fecal contamination of food and water (O'Leary, 1989). Most strains are regarded as innocuous commensals; however, some strains can produce toxins and cause disease (Doyle et al., 1997). Toxin producing strains of *E. coli* have been classified into five virulence groups including enteroaggregative (EAggEC), enteroinvasive (EIEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), and enterohemorrhagic (EHEC) (Jay, 1996).

Escherichia coli O157:H7, which is identified by its O (157) and H (7) antigens, is classified as EHEC based on the production of shiga-like toxins and the ability to cause certain disease symptoms (Buchanan and Doyle, 1997). Shiga-like toxins are verotoxins similar to those produced by *Shigella dysenteriae* (O'Brien and Holmes, 1987). Although there are more than 60 strains within the EHEC group that produce shiga-like toxins, *E. coli* O157:H7 is the most significant pathogen and the serotype most often associated with human illness (Feng, 1995).

Serotype O157:H7 is similar to most other *E. coli* strains, with a few notable differences. Unlike other *E. coli* strains, most O157:H7 strains are unable to ferment

sorbitol within 24 hours and are negative for β-glucuronidase (Ratnam et al., 1988). Strains capable of fermenting sorbitol have been identified; however, and it is interesting to note that the sorbitol negative phenotype is not associated with virulence (Fratamico et al., 1993). Another difference is the inability of serotype O157:H7 to grow well at 44 - 45.5^{o} C, which is the temperature range commonly used for the isolation of *E. coli* from foods (Doyle and Schoeni, 1984).

2. Illness

E. coli O157:H7 was first acknowledged as a human pathogen in 1982 after being linked to outbreaks of hemorrhagic colitis associated with the consumption of ground beef (Riley, 1987). Since then, it has become recognized as a significant cause of foodborne illness (Doyle, 1991). The advent of *E. coli* O157:H7 has caused much concern due to the potential severity of the disease syndromes associated with it and the fact that it has a cattle reservoir (Bean and Griffin, 1990).

Illness due to *E. coli* O157:H7 is usually severe and is expressed as one of three syndromes including hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP) (Padhye and Doyle, 1992). Symptoms of HC typically begin with severe abdominal cramps and watery diarrhea followed by nausea and vomiting. Within days most, but not all, patients develop profuse bloody diarrhea. The average length of illness is eight days and the disease is usually self-limiting; however, serious complications and deaths have occurred (Riley, 1987). HUS

is manifested by sudden onset of bloody diarrhea followed by hemolytic anemia, thrombocytopenia, and acute renal failure (Padhye and Doyle, 1992). Patients usually require dialysis and blood transfusions and may develop diseases of the central nervous and cardiovascular systems that may result in death (Doyle, 1991). TTP typically consists of microangiopathic hemolytic anemia, thrombocytopenia, neurologic disorder, and fever. Persons with TTP often develop cerebral blood clots, frequently leading to death (Padhye and Doyle, 1992).

3. Reservoirs and Disease Transmission

The main reservoir for pathogenic *E.coli* is the intestinal tract of warm-blooded animals (Olsvik et al., 1991). More specifically, cattle are commonly regarded as the primary reservoir for *E. coli* O157:H7 (Borczyk et al., 1987). Research has shown that the bacteria are often distributed through a common source such as drinking water on farms and that strains may remain in a given herd for up to two years (Shere et al., 1998). *E. coli* O157:H7 is capable of survival in bovine feces for up to 56 days and thus, feces may play an important role in the spread of this pathogen (Wang et al., 1996). *E. coli* O157:H7 is more prevalent in younger cattle than adults and the level in calf feces may be as high as 10^5 CFU/g (Zhao et al., 1995). The organism is confined to the gastrointestinal tract of cattle and does not cause illness in them (Brown et al., 1997). Other animals including deer (Keene et al., 1997), sheep (Kudva et al., 1996), and birds (Wallace et al., 1997) have also been identified as carriers of *E. coli* O157:H7 and these

may play an important role in the transmission of disease.

Transmission of disease from *E. coli* O157:H7 may occur through person-to-person contact, typically in day care centers (Belongia et al., 1993), and contaminated water (Doyle et al., 1997). Both recreational water, where contaminated water is ingested while swimming (Keene et al., 1994), and drinking water (Swerdlow et al., 1992) have been implicated. However, the majority of outbreaks result from the consumption of contaminated foods, in particular, improperly cooked beef, dairy products, and produce (Borczyk et al., 1987; Griffen and Tauxe, 1991).

4. Infective Dose and Susceptible Populations

Analyses of foods associated with outbreaks of illness due to *E. coli* O157:H7 have indicated that the infective dose may be very low (Doyle et al., 1997). The ease with which the bacteria can be spread through person-to-person transmission also suggests that the number of organisms required to cause illness is low (Lior, 1994).

All age groups are susceptible to illness from *E. coli* O157:H7; however, serious illness is most frequently seen among infants, small children, and the elderly (Doyle et al., 1997). HUS is most common among children less than ten years old (Buchanan and Doyle, 1997). The incidence of TTP is infrequent and appears most often in adults (Padhye and Doyle, 1992).

5. Pathogenicity

Although the exact mechanism of pathogenicity has not been defined, many elements have been associated with the virulence of *E. coli* O157:H7. The most often cited factor is the production of verotoxins. All strains designated as EHEC form Shiga toxin 1 (SLT-I) and/or Shiga toxin 2 (SLT-II) which are proteins targeted to endothelial cells of the colon and renal glomeruli (Buchanan and Doyle, 1997). Under new terminology, SLT-I has been renamed Stx1 and SLT-II has been designated Stx2 (Jay, 1996). The capability for toxin production is believed to have been acquired by way of a bacteriophage either indirectly or directly from *Shigella* (Buchanan and Doyle, 1997). Shiga and Shiga-like toxins bind to receptors in the host's intestinal mucosal cells and are believed to obstruct protein synthesis, leading to death of the cell (O'Brien and Holmes, 1987).

Besides SLT-I and SLT-II, variant verotoxins have been described and may play a role in causing disease (Padhye and Doyle, 1992). Another factor that may be associated with the pathogenicity of *E. coli* O157:H7 is its ability to adhere to intestinal cells and subsequently to colonize the intestinal tract, where toxin production commences (Padhye and Doyle, 1992).

6. Factors Affecting Growth and Survival

It has been shown that *E. coli* O157:H7 is not particularly resistant to heat. Doyle and Schoeni (1984) found it to be more sensitive to heat than salmonellae when inoculated into ground beef. The heat resistance of a particular organism is affected by

multiple factors including cell age, the stage of growth, growth temperature, growth medium, and nature of the medium in which heating occurs (ICMSF, 1980). Different strains of *E. coli* O157:H7 show variations in heat resistance (Semanchek and Golden, 1998). In addition, high concentrations of solutes (Splittstoesser et al., 1996) and fats (ICMSF, 1980) in foods can afford some protection from heat.

E. coli O157:H7 is capable of surviving for extended periods at sub-freezing temperatures. Doyle and Schoeni (1984) found that it survived in ground beef stored at -20° C for nine months with little decrease in number. Likewise, Semanchek and Golden (1998) saw a decrease of from 4 to 6 log CFU/ml in cultures in peptone water stored at -20° C for seven months; however, substantial numbers of viable cells were still recovered. Enhanced survival at 4-8° C has also been documented in TSB (Clavero and Beuchat, 1996), water (Wang and Doyle, 1998), and cider (Zhao et al., 1993).

As stated previously, *E. coli* O157:H7 does not grow well, if at all, at temperatures normally used for the recovery of *E. coli* from foods. The organism has been found to grow well in Trypticase soy broth (TSB) between 30° C and 42° C with an optimum of 37° C (Doyle and Schoeni, 1984). However, it is capable of growth over a wide range of temperatures. An increase of 1 to 2 log CFU/ml in milk stored at 8° C and of 3 to 5 log CFU/ml in milk at 15° C has been documented (Wang et al., 1997). Rajkowski and Marmer (1995) found that growth can occur in Brain heart infusion broth (BHI) at temperatures as low as 8° C if allowed sufficient incubation time. They also found that the organism can grow at fluctuating temperatures and that fluctuation often

resulted in more rapid growth.

The effect of water activity on the survival and growth of *E. coli* O157:H7 has most often been studied in terms of the effect of NaCl. Glass et al. (1992) studied the growth of *E. coli* O157:H7 in TSB amended with NaCl and found that it grew well in broth with NaCl concentrations of up to 2.5% and is capable of growth at concentrations of 6.5%. However, concentrations >8.5% were inhibitory. Clavero and Beuchat (1996) found that populations of up to 2 log CFU/g in salami at a_w s of .95 and .99 did not survive storage of 32 days. However, the researchers noted that higher populations would likely survive for longer periods, especially at a storage temperature of 5 ^{o}C.

The one environmental parameter most extensively studied in relation to its effects on the survival and growth of *E. coli* O157:H7 is pH. Unlike most foodborne pathogens, *E. coli* O157:H7 has the unique ability to tolerate acidic conditions (Doyle et al., 1997). Gorden and Small (1993) studied acid resistance in enteric pathogens and found that unlike *Salmonella*, some *Shigella* species and strains of *E. coli* can tolerate a pH of 2.5 for short periods. It has been suggested that the ability of pathogens like *E. coli* O157:H7 to survive in such acidic environments is a contributing factor in their virulence, allowing a small number of cells to cause illness due to survival in the gastric tract (Leyer et al., 1995).

Benjamin and Datta (1995) reported 100% survival of *E. coli* O157:H7 strain 43895 and survival to a lesser degree of several other strains for at least five hours at a pH of 2.5. Populations of *E. coli* O157:H7 strains 43895 and 43889 were reduced by

just 1 log after 24 hours in pH 2.0 TSB while a control strain of *E. coli* was not detectable after 3 hours (Miller and Kaspar, 1994). In addition, survival under acidic conditions for extended periods has been documented. Conner and Kotrola (1995) reported survival for up to 56 days at pH values as low as 4.0.

Although growth is generally unaffected at pH values as low as 5.5, more acidic conditions dramatically reduce growth rates (Buchanan and Doyle, 1997). Nevertheless, *E. coli* O157:H7 has been found to be capable of growth under certain conditions at lower pH values. Glass et al. (1992) documented growth in TSB acidified to pH 4.5 with HCL and to pH 4.6 when lactic acid was used as the acidulant. Likewise, Conner and Kotrola (1995) observed growth in TSB acidified with citric acid at pH values as low as 4.0.

Research indicates that the growth and survival of *E. coli* O157:H7 under acidic conditions are dependent on many factors including the type and concentration of acid, temperature, and water activity (Buchanan and Doyle, 1997). Organic acids have been shown to enhance survival when compared with that in unacidified controls; however, survival is influenced by both temperature and type of acidulant (Conner and Kotrola, 1995). Survival at low pH is typically greater at 4^{o} C than at 25^{o} C (Miller and Kaspar, 1994).

7. Mechanisms of Acid Resistance

Although the exact mechanism responsible for the acid tolerance of *E. coli*

O157:H7 is not known, it seems to be associated with proteins that can be triggered by prior exposure to acidic conditions (Doyle et al., 1997). Studies have revealed that acid tolerance is dependent on the growth phase and the pH of the growth medium (Benjamin and Datta, 1995; Gorden and Small, 1993). Arnold and Kaspar (1995) found that stationary-phase and starved log-phase cells were more acid tolerant than mid log-phase cells. In addition, Benjamin and Datta (1995) reported that the pH of the growth medium significantly affected acid resistance such that the higher the growth medium pH, the lower the tolerance and vice versa. Lin et al. (1996) studied three mechanisms of acid resistance including oxidative, arginine-dependent, and glutamate-dependent and proposed that multiple acid resistance systems contribute to the ability of *E. coli* O157:H7 to survive under differing conditions of acid stress. They also found that the acid resistance systems can stay active for prolonged periods of storage at 4°C.

Acid tolerance systems can be induced by exposure of the bacteria to sublethal levels of certain stress conditions (Goodson and Rowbury, 1989). When the stress is low pH, this phenomenon is often termed acid adaptation. Leyer et al. (1995) observed increased resistance to organic acids and enhanced survival during sausage fermentation and in acidic foods, when *E. coli* O157:H7 was adapted to acid by prior exposure to mildly acidic conditions (pH 5.0). Similar results were also reported by other researchers (Brudzinski and Harrison, 1998; Garren et al., 1997). In addition, Garren et al. (1997) reported enhanced survival of acid shocked cells when compared with that of acid adapted cells upon subsequent exposure to pH 3.5 and 4.0. Acid shocked cells were

previously exposed to more acidic conditions than acid adapted cells.

8. Foodborne Outbreaks of Illness

E. coli O157:H7 was a relatively unknown serotype at the time of the first acknowledged outbreaks in 1982. These outbreaks occurred in Oregon and Michigan and involved consumption of contaminated ground beef (Riley, 1987). The majority of outbreaks have been linked to the consumption of improperly cooked beef (Borczyk et al., 1987; Griffen and Tauxe, 1991). In 1993, in what has proven to be one of the largest outbreaks, a multistate outbreak in the pacific northwest was linked to the consumption of contaminated ground beef (CDC, 1993).

Aside from ground beef, several other foods have been implicated in *E. coli* O157:H7 outbreaks worldwide. These include unpasteurized milk, unpasteurized apple juice and cider, salami, lettuce, sprouts, yogurt, prepared sandwiches, and water (Buchanan and Doyle, 1997).

9. Survival in Various Food Products

Because of the seriousness of the syndromes attributed to *E. coli* O157:H7 and the wide range of food products associated with outbreaks, much research has focused on the survival and growth of the organism in various foods and beverages. Wang et al. (1997) demonstrated that the organism can survive for up to 28 days in milk with growth occurring at temperatures as low as 8^{o} C. Survival in water for up to 300 days has also

been documented (Warburton et al., 1998) with survival greatest at 8°C (Wang and Doyle, 1998).

Because produce is often consumed without any heat treatment, much attention has been given to the survival and growth of *E. coli* O157:H7 in these foods. Abdul-Raouf et al. (1993) studied the effects of modified atmospheric packaging, storage temperature, and time on growth and survival in salad vegetables and found that the organism is capable of growth under conditions used in commercial production. Del Rosario and Beuchat (1995) documented growth on the rinds and cut cubes of cantaloupe and watermelon stored at 25° C. Survival on radish sprouts has also been reported (Itoh et al., 1998).

The ability of *E. coli* O157:H7 to survive under acidic conditions has called attention to the survival of the pathogen in various acidic food products that rely in part on their acidity for preservation. Among the products that have been evaluated are fermented and dried sausages, acidic condiments, and apple juice or cider. Clavero and Beuchat (1996) noted survival for at least 32 days in salami inoculated after processing and held at 5°C. In another study where sausage was inoculated prior to fermentation to a pH of 4.8 and subsequently dried, vacuum packed, and stored at 4°C, survival with a reduction of just 2 logs was observed after two months (Glass et al., 1992). Survival during the manufacture of pepperoni has also been reported (Riordan et al., 1998).

Acidic condiments evaluated for their potential to support the growth and/or survival of *E. coli* O157:H7 include mayonnaise, salad dressings, ketchup, mustard,

relish, and soy sauce. Several studies have found that growth is not supported in mayonnaise or mayonnaise-based dressings but that the organism is capable of survival in these products with greater survival at refrigerated temperatures (Hathcox et al., 1995; Raghubeer et al., 1995; Zhao and Doyle, 1994). Erickson et al. (1995) studied survival of *E. coli* O157:H7 at 25° C in commercial mayonnaise products and observed a reduction of at least 7 logs in <1 to 3 days in products with a pH <3.6. Raghubeer et al. (1995) reported that *E. coli* O157:H7 survived longer in refrigerated ranch salad dressing than in mayonnaise, possibly due to differences in pH, water activity, nutrients, and the presence of lysozyme in mayonnaise. Tsai and Ingham (1997) discovered that *E. coli* O157:H7 was reduced to undetectable numbers in mustard and relish stored at 5 and 23° C after just one hour. In the same study, however, the pathogen survived in ketchup for up to seven days with greater survival seen in acid adapted cells and at 5° C. Survival in soy sauce follows a similar pattern as in other condiments with greater numbers surviving for longer periods at lower temperatures (Masuda et al., 1998).

Other acidic foods studied include yogurt and various types of cheeses. In yogurt containing live cultures and at a pH of 4.47, *E. coli* O157:H7 survived for 17 days at both 4 and 10° C. The pathogen was also able to survive during the manufacture of Colby, Romano, and Feta cheeses for 27 to 30 days (Hudson et al., 1997). The survival and growth of *E. coli* O157:H7 in apple juice and cider will be covered in section C.

10. Methods for Detection and Isolation from Foods

Differences between *E. coli* O157:H7 and most other *E. coli* strains have been mentioned in section 1. Such differences can be used to an advantage when isolating *E. coli* O157:H7 from foods. The inability to ferment sorbitol within 24 hours is regarded as a phenotypic characteristic and therefore, sorbitol containing media such as sorbitol MacConkey agar (SMAC) are used to differentiate *E. coli* O157:H7 from other related bacteria (Padhye and Doyle, 1992). However, a small percentage of *E. coli* O157:H7 are sorbitol positive (Fratamico et al., 1993) and a few other *Escherichia* strains are sorbitol negative (Padhye and Doyle, 1992), thus the possibility of false-positive and false-negative isolates exists. Another characteristic that can be used to differentiate *E. coli* O157:H7 from other enterics is the fact that it is negative by the MUG assay (Doyle and Schoeni, 1984).

Because of the apparent low infective dose for *E. coli* O157:H7, detection of low numbers is important and this often requires enrichment media. Some types of enrichment media that have been employed for this purpose are mTSB (Doyle and Schoeni, 1987) and dm TSB-CA (Padhye and Doyle, 1991). It is also often necessary to consider the recovery of injured cells which may not be detected using selective media, but could cause illness. Several different repair procedures significantly increase detection of acid injured cells when compared with that of SMAC; however, the best detection is obtained using the nonselective media TSA (Silk and Donnelly, 1997). When testing for injured cells in previously frozen apple cider, Sage and Ingham (1998) saw increased detection over SMAC while using the hydrophobic grid membrane filter-

SD-39 agar method; however, spread plating on TSA resulted in better recovery.

Once isolated, suspected *E. coli* O157:H7 colonies can be confirmed using biochemical and serological tests. Since *E. hermanii* and other enterics are serologically similar to *E. coli*, biochemical tests are necessary to eliminate false-positive agglutination tests (Vernozy-Rozand, 1997). Isolates which have been confirmed as *E. coli* through biochemical testing can be confirmed serologically as O157:H7 by using O and H antisera (Vernozy-Rozand, 1997). For the isolation of *E. coli* O157:H7 from foods that have not been artificially inoculated, it is also important to confirm the presence of SLT or SLT genes (FDA, 1995).

B. Apples and Other Produce

1. General

In many parts of the world, apples (*Malus* X *domestica* Borkh.) are an important agricultural crop and are processed into a variety of products. The United States ranks second in world apple production and apples are the third most important fruit crop grown in the U.S. (Downing, 1989). In terms of per-capita consumption, apples rank third following citrus fruits and bananas (Downing, 1989). Although the majority of apples produced in the U.S. are consumed fresh (55-60%), approximately 21% is processed into juices including apple cider and the remainder is processed into a variety of products including canned sauces, jellies and related products, vinegars, and canned, dried, frozen, and fresh slices (U.S. Apple Association, 1999).

2. Microbiology

Apple microflora comes from two main sources. The primary or resident microflora, which adhere to the surface and remain relatively constant, and the secondary microflora or that which comes from external contamination (Doores, 1983). Due to the composition and pH of apples, the resident microflora of sound apples is composed primarily of yeasts, molds, acetic acid bacteria, and lactic acid bacteria (Doores, 1983). Secondary microflora can come from various sources including soil, irrigation water, dust, animals and insects, human handling, transport and storage equipment, wash water, and processing equipment and facilities (Beuchat, 1995). Both the types and amounts of primary and secondary microflora present on apples vary with geography, climate, cultivars, and processing conditions (Doores, 1983).

Yeasts and molds are the predominant resident organisms present with typical total counts ranging from 10^2 to 10^6 per apple for yeasts and 10^3 to 10^5 for molds (Doores, 1983). Both can cause significant spoilage problems with processed products; however, with the exception of patulin, they do not usually result in unsafe products (Downing, 1989). Lactic and acetic acid bacteria are present in much lower numbers than yeasts and molds and, like yeasts and molds, can lead to spoilage (Downing, 1989).

3. Contamination of Produce by Pathogens

Bacterial pathogens on produce including *Shigella*, *Salmonella*, and *E. coli* O157:H7, in contrast to spoilage organisms, have led to serious illness (Beuchat, 1995).

However, the percentage of foodborne disease outbreaks due to the consumption of fruits and vegetables (5%) is low in comparison to foods such as meat and seafood products. In addition, many of the outbreaks attributable to produce have been associated with *Clostridium botulinum* contamination of home canned vegetables (Bean and Griffin, 1990). Nevertheless, foodborne infections due to both raw and processed fruit and vegetables do occur.

Contamination of fruits and vegetables by pathogens often originates from agricultural practices such as irrigation with polluted water or fertilization with manure (FDA, 1998a; Nguyen and Carlin, 1994). In addition, soil dwelling pathogens such as *C. botulinum* and *Listeria monocytogenes* are often isolated from produce (Madden, 1992). The possibility for contamination of fruits and vegetables with pathogens is high due to exposure to various conditions during growing, harvesting, and transport (Madden, 1992). Other sources of pathogens are human handlers, transport containers and machinery, processing equipment and facilities, and processing water (Beuchat, 1995; FDA, 1998a). According to Brackett (1992), processing steps such as cutting and peeling often result in increased levels of contamination. In addition, processing steps such as washing, which are intended to decrease contamination, can also lead to increased levels of contamination by spreading contaminants over produce (FDA, 1998a). Since processing water can be a source of potential pathogens, the microbiological quality of water used in processing is very important (Geldreich and Bordner, 1971).

Fruits have had a relatively good track record when it comes to foodborne illness. This may be due in part to their natural defense mechanisms including a thick, waxy skin, natural antimicrobial constituents, and organic acids that result in a low pH (Doyle, 1990). However, bacteria can penetrate the exterior through breaks in the skin introduced during growing, harvesting, handling, and processing (Doores, 1983).

The level of spoilage in produce is often directly proportional to the number of microorganisms present (Nguyen and Carlin, 1994). Damaged, rotten, molding, or otherwise spoiled, produce should be separated from that which is sound prior to washing and other processing steps since it may harbor bacteria that could be spread through wash water to sound apples (Beuchat and Ryu, 1997).

4. Growth and Survival of Pathogens in Produce

Temperature, atmospheric conditions, interactions with other organisms, and the nature of plant tissue involved can all affect the growth and survival of pathogens on produce (Nguyen and Carlin, 1994). In general, as storage temperature increases, so will microbial growth. Refrigeration tends to slow the growth of bacteria however, some pathogens can survive at cold temperatures (Brackett, 1992). Growth and survival of such pathogens as *Shigella sonnei*, *L. monocytogenes*, and *Aeromonas hydrophila* on produce stored under refrigeration has been documented (Nguyen and Carlin, 1994). The atmospheric conditions under which produce is stored and packaged is often modified to prevent the growth and survival of pathogens (Nguyen and Carlin, 1994).

The storage life of apples can be extended greatly by using controlled atmosphere storage, however this procedure is usually employed to limit the growth of spoilage organisms rather than pathogens (Downing, 1989).

The nature of the plant tissue itself may play the greatest role in influencing the growth and survival of pathogens on produce. As mentioned previously, breaks in the protective skin can allow for the penetration of pathogens. Other natural defense mechanisms exist under the skin however. Organic acids present within many fruits, due to their effect on pH, can act to inhibit the growth of many microorganisms (Davidson and Branen, 1993). In addition, many naturally occurring antimicrobial substances, such as essential oils and other compounds, present in some fruits and vegetables can affect bacterial growth and survival (Davidson and Branen, 1993; Nguyen and Carlin, 1994).

Despite these natural defense mechanisms, pathogens can and do survive within the tissue of produce. In fact, when intact apples are inoculated with *E. coli* O157:H7 the greatest level of contamination is associated with the outer core region followed by the skin (Buchanan et al., 1998). Surveys have been conducted in which pathogens such as *L. monocytogenes*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *E. coli*, and *Salmonella* have been isolated from various types of produce (Nguyen and Carlin, 1994). As for the survival and growth of *E. coli* O157:H7 in apples, research has been limited. Abdul-Raouf et al. (1993) demonstrated the ability of *E. coli* O157:H7 to grow on raw vegetables subjected to commercially used processing and storage conditions. Fisher and Golden (1998) reported that this pathogen could survive for up to 18 days in ground

apples stored at 4° C and that survival was probably influenced by more than just pH.

5. Preventing Contamination of Produce

Preventive measures should begin in the field and orchard since agronomic practices can greatly affect the microbiological quality of produce. The use of uncomposted manure to fertilize orchards and fields and allowing domestic and wild animals to frequent growing areas can increase the chances of contamination with pathogens through fecal matter (Brackett, 1992, FDA, 1998a). The microbiological quality of irrigation water should be addressed (FDA, 1998a). In addition, it is necessary to consider harvesting practices. Worker sanitation as well as the condition of harvesting equipment and transportation machinery are important factors (Brackett, 1992, FDA, 1998a).

Measures to prevent contamination should continue inside the processing plant. Good employee and overall plant sanitation are necessary to help insure the microbiological safety of produce (Hurst and Schuler, 1992). The microbiological quality of cleaning and processing water is also very important and coliform testing is required for most water supplies used for food processing (Gould, 1994). Every step involved in processing should be considered a potential source of contamination. Unit operations such as cutting, slicing, and peeling allow microorganisms access to internal nutrients and increase the surface area available for growth (Brackett, 1992). Temperature control will help to maintain product quality while slowing microbial

growth (FDA, 1998a).

6. Cleaning and Disinfection of Produce

The purpose of cleaning produce is to remove field soil, pesticide residues, insects, microorganisms, and other extraneous matter prior to further processing (Gould, 1996). A typical procedure for washing apples employs water or chlorinated water, may also include some means of scrubbing, and is intended for the removal of field soil prior to processing (Downing, 1989). According to Beuchat (1992), washing fruits and vegetables by means of flumes or sprays can help to reduce surface microbes however, complete sterilization cannot be achieved.

As mentioned previously, washing may lead to increased levels of contamination if contaminated water is used or if wash water is recycled. In addition, for certain types of produce, washing in a solution that is colder than the produce can lead to internalization of bacteria on the surface due to a pressure differential (FDA, 1998a). *Salmonella montevideo* infiltration in tomatoes is significantly greater when warm tomatoes are immersed in cold water than when the temperature of tomatoes is equal to or less than that of the water (Zhuang et al., 1995). The frequency and degree of internalization is greater when warm apples rather than cold are immersed in cold water containing *E. coli* O157:H7 (Buchanan et al., 1998).

Although washing with water alone can be effective in removing field soil, its effectiveness for the removal of surface bacteria has been questioned. Several studies

have demonstrated the inability of water washes to remove bacteria from the surface of fruits and vegetables (Brackett, 1987; Harmon et al., 1987; Nguyen and Carlin, 1994; Shapiro and Holder, 1960).

An important distinction must be made between cleaning and sanitizing. Cleaning has been defined as the physical removal of soil while sanitizing refers to the removal or inactivation of microorganisms by means of chemical, heat, or some other treatment (Marriott, 1994). Although treatments such as heat, ozone, and irradiation have been used successfully, they may not be suitable for use with produce in some instances. Heating may be appropriate for produce intended for further processing and many types of fruits and vegetables are blanched before processing (ICMSF, 1980). Ozone has good antimicrobial activity; however, it can also cause injury to produce. Likewise, irradiation has been used effectively on a variety of foods but it is not approved for use in many cases (Beuchat, 1992).

Chemical sanitizing agents include halogens, organic acids, iodophors, quaternary ammonium compounds, and peroxy compounds (Davidson and Branen, 1993). However, not all of these compounds have been approved for use on produce. The FDA has identified the kinds and concentrations of chemicals permitted for use; however, usage often varies with the type of produce. Usually, such chemicals are either those substances considered generally recognized as safe (GRAS) in foods or those approved by prior authorizations to be safe for washing produce (FDA, 1998b).

Among the chemicals used most frequently in the food industry are chlorine

compounds, typically as hypochlorites (Davidson and Branen, 1993). The antimicrobial activity of hypochlorite can be affected by several factors including pH, temperature, organic load, water hardness, and the concentration of the solution (Block, 1977). Chlorine is believed to act on cells through its effects on the cell membrane and DNA and through the inhibition of certain enzymes (Davidson and Branen, 1993).

Hypochlorites are widely added in concentrations of 50 to 100 ppm to solutions used to wash, cool, and transport fruits and vegetables (Eckert and Ogawa, 1988). Chlorine solutions of comparable strength have proven to be effective in some situations. Dipping tomatoes in a 50 ppm solution of chlorine for two minutes significantly reduces surface populations of *A. hydrophila* (Velazquez et al., 1998). Populations of *E. coli* O157:H7 in apples are reduced by 1-3 logs when apples are immersed in 2000 ppm hypochlorite for 1 minute; however, the treatment does not eliminate the bacteria, particularly that located in the outer core region (Buchanan et al., 1998). Several researchers have reported less than ideal results when using hypochlorite solutions as antimicrobial wash treatments for various fruits and vegetables (Adams et al., 1989; Beuchat and Brackett, 1990; Brackett, 1987; Golden et al., 1987; Wei et al., 1995; Zhang and Farber, 1996; Zhuang et al., 1995).

Research has shown that the antimicrobial effectiveness of chlorine is best when the level of background microflora on produce and organic material in solution is low, and when the pH of the solution is approximately 5.0 (Adams et al., 1989). However, these factors are often difficult to control under processing conditions (Garg et al., 1990).

The microbial counts of fruits and vegetables and thus, the organic load in wash and flume water is usually high (Nguyen and Carlin, 1994). In addition, wash solutions are often recycled leading to a higher organic load and a greater chance of contamination (Brackett, 1992).

Organic acids such as acetic, citric, and lactic are also known to possess antimicrobial activity and have been used in various applications (Davidson and Branen, 1993). It is believed that organic acids act on microbial cells by lowering the pH of the environment causing disruption of membrane functions and the functions of key enzymes (Dillon and Cook, 1994). The effectiveness of organic acids varies with the type of acid, concentration of the solution, and the amount of undissociated acid in solution. Since the undissociated acid is primarily responsible for the antimicrobial activity, it is best to use a particular acid at or below its $pk_{a.}$ This is the pH at which the concentration of undissociated acid is maximized (Davidson and Branen, 1993).

Researchers have seen varying success when using various organic acid solutions as spray or dip treatments to remove microorganisms from produce and meats. Small reductions in *E. coli* O157:H7 on beef are obtained using acetic, lactic, and citric acids as spray treatments at concentrations up to 5%; however, complete elimination is not achieved (Conner et al., 1997; Cutter and Siragusa, 1994). *L. monocytogenes* reductions of less than one log occur when lettuce and cabbage is treated by dipping in 1% solutions of acetic and lactic acids (Zhang and Farber, 1996).

Other studies have shown greater success in reducing microbial contamination.

Shapiro and Holder (1960) reported good reductions in total bacterial counts when using citric and tartaric acids as dip treatments for packaged salad greens. A ten percent acetic acid solution causes complete inactivation of *E. coli* O157:H7 cultures on agar in one minute and concentrations as low as 0.1% inhibit growth (Entani et al., 1998). Karapinar and Aktug Gonul (1992) achieved a 5 log reduction of *Yersinia enterocolitica* on parsley by dipping in both 2% and 5% acetic acid for 15 minutes. *E. coli* O157:H7 is reduced by 2 logs on broccoli and 3 logs on tomatoes with little effect on quality when acetic acid solutions of 2% and 5% are applied as a dip treatment (Peters, 1995).

Both inorganic and organic peroxy compounds are strong oxidizing agents that are capable of antimicrobial activity (Davidson and Branen, 1993). Hydrogen peroxide, the most widely used of the inorganic peroxides, is believed to exert its effects on the bacterial cell through the production of a hydroxyl free radical which is a powerful oxidant (Block, 1977). Of the organic peroxides, peroxyacetic acid is the most commonly used. Its antimicrobial activity is likely due to denaturation of proteins, inactivation of enzymes, and disruption of membranes and cellular transport (Davidson and Branen, 1993). An advantage to the use of peroxy compounds is that their breakdown products are nontoxic substances such as water, oxygen, and acetic acid (Davidson and Branen, 1993)

Hydrogen peroxide has been shown to be effective as a disinfectant for poultry and produce. When used in poultry chiller water, hydrogen peroxide was an effective bactericide; however, it also lead to bleaching and bloating of carcasses (Lillard and

Thomson, 1983). Hydrogen peroxide is also effective in inhibiting bacterial blotch development in mushrooms and extending the shelf-life of fresh-cut produce (Sapers and Simmons, 1998). A decrease in *E. coli* O157:H7 of 2 logs on broccoli and 4 logs on tomatoes was achieved using 3% hydrogen peroxide applied as dip or spray treatments. Even greater effectiveness is seen when 5% acetic acid is followed by 3% hydrogen peroxide (Peters, 1995).

Little research exists on the use of peroxyacetic acid to disinfect food products. It is often used for clean-in-place sanitizing in beverage and dairy plants due to its effectiveness against yeasts and molds (Marriott, 1994). It has also been used as a sanitizer for food contact surfaces and has been found to be effective for the inactivation of various pathogens including *L. monocytogenes*, *Y. enterocolitica*, and *Campylobacter jejuni* (Davidson and Branen, 1993).

C. Apple Cider

1. General

Although a legal definition for cider does not exist, in the United States the term cider is used to describe the unfermented and oxidized juice of freshly pressed apples that contains some or all of the suspended solids. Apple juice, on the other hand, is usually not oxidized, is often clarified, and usually receives a heat treatment (Downing, 1989). In most other countries, apple cider usually refers to the fermented juice of apples. This product is usually called hard cider in the United States (Harris, 1997). The

sugar content of apple cider is typically between 10.1 and 16.9° brix and its pH ranges between 3.31 and 4.24 (Mattick and Moyer, 1983).

Approximately 21% of the annual U.S. apple crop is typically processed into juice including apple cider (Binnig and Possmann, 1993; U.S. Apple Association, 1999). Among fruit juices, apple juice consumption ranks second to orange juice in the U.S. (Downing, 1989). Before the 1940's, most apple juice was consumed fresh in the form of cider and was mainly a seasonal beverage (Downing, 1989). Today, pasteurized apple juices that are canned and bottled predominate (Downing, 1989). However, apple cider has its own distinctive appeal, is desired by many consumers, and provides an important source of income for apple growers (USDA, 1977). In the U.S., there are approximately 9,000 apple growers with orchards covering over 459,000 acres (U.S. Apple Association, 1999).

2. Production

Cider production begins with the growing and selection of apples. Apples for cider production may be grown by the processor or obtained from outside sources. Usually, cider producers blend the juices from several cultivars in order to achieve the desired equilibrium between acidity, sweetness, aroma, and mouthfeel (Downing, 1989). The composition and thus the sensory characteristics of cider varies with the geographic region, the choice of cultivars, and the maturity of apples (The Pennsylvania State University, 1998).

To obtain the best quality, apples for cider should be clean, ripe, undamaged, and free from decay (Downing, 1989). Once inside the processing facility, apples are typically sorted before further processing to remove damaged, rotten, or otherwise unacceptable fruit (Binnig and Possmann, 1993). Apples selected for processing are then cleaned to remove field soil, pesticide residues, insects, microorganisms, and other extraneous matter (Gould, 1996).

Sorted and cleaned apples are first ground to a mash and then pressed to extract the juice (Downing, 1989). The freshly pressed juice is then strained to remove coarse particles and extraneous matter and pumped to a refrigerated storage tank to allow sedimentation to take place (USDA, 1977). Cider may then be pumped from above the sediment, filtered if desired, and bottled (The Pennsylvania State University, 1998).

3. Preservation

While apple juice usually receives some form of heat treatment, cider producers have historically relied upon the products inherent acidity as well as refrigeration, and chemical preservatives for preservation (Downing, 1989). However, until recently, preservation measures were viewed solely as a means to prevent fermentation by yeasts and spoilage by molds, lactic acid bacteria, and *Acetobacter* (Doores, 1983). The most commonly used preservation measures are refrigeration, freezing, pasteurization, and the addition of chemical preservatives (USDA, 1977).

Prompt refrigeration of cider will help to retain the best flavor and can prevent

fermentation and spoilage for a few weeks. Freezing can extend the shelf life for even longer periods and has made year round consumption of cider a reality (Downing, 1989). Chemical preservatives such as potassium sorbate and sodium benzoate can also help to retard fermentation and spoilage and their effectiveness is enhanced when used in conjunction with refrigeration (USDA, 1977). The fourth preservation measure, pasteurization will be covered later in this section.

4. Outbreaks of Foodborne Illness

As mentioned previously, in the past, there was little concern about foodborne illness from high acid foods such as apple cider. Indeed, between 1923 and 1974, only two documented outbreaks of foodborne illness were linked to the consumption of cider. These outbreaks involved contamination with *S. typhi* and *S. typhimurium* (Parish, 1997). Between 1973 and 1987 illness associated with the consumption of fruit juices was still relatively rare with only 2% of documented outbreaks attributed to nondairy beverages (Bean and Griffin, 1990).

Beginning in the early 1980's, however, a new pathogen began to be associated with unpasteurized apple cider and juices. An early outbreak of HUS associated with apple juice or cider occurred in Canada in 1980, two years before the recognition of *E. coli* O157:H7 as a foodborne pathogen. However, the agent responsible for the illness was not identified, possibly because of delays between sampling and analysis (Steele et al., 1982). Outbreaks of HUS in Massachusetts in 1991 (Besser et al., 1993) and

Connecticut in 1996 (CDC, 1997) attributed to *E. coli* O157:H7 were associated with drinking contaminated apple cider. Unpasteurized cider has also been implicated in outbreaks of cryptosporidiosis (CDC, 1997; Millard et al., 1994). In a 1996 multistate outbreak involving unpasteurized apple juice in the pacific northwest, Odwalla brand apple juice and juice mixtures contaminated with *E. coli* O157:H7 were implicated and caused a nationwide recall (CDC, 1996).

5. Survival of *E. coli* O157:H7 in Apple Cider

The outbreaks mentioned above, and the results of research inspired by them, have generated much concern about the safety of unpasteurized apple cider. Goverd et al. (1979) sampled cider from several manufacturers and found that several Salmonella serotypes and some coliforms were capable of survival at pH values as low as 3.4. Silk et al. (1997) sampled cider from twelve plants and reported that all 59 samples contained coliforms despite the fact that the average pH of the samples was 3.34.

Many studies have proven the ability of *E. coli* O157:H7 to survive in cider despite its low pH and regardless of whether refrigeration or preservatives were used. Miller and Kaspar (1994) reported survival of *E. coli* O157:H7 for at least 24 hours in cider adjusted to a pH of 2.0. They also saw enhanced survival in cider at 4 °C when compared with survival at 25° C, especially at lower pH values. Survival was not affected by potassium sorbate or sodium benzoate. Likewise, Zhao et al. (1993) reported longer survival times at 8° C than at 25° C, regardless of whether either preservative was

used. As mentioned previously, prior exposure to mildly acidic environments enhances survival at more extreme pH values. Acid adapted *E. coli* O157:H7 cells survived in pH 3.4 cider for up to 81 hours while nonadapted cells were not detected after 28 hours (Leyer et al., 1995)

Freezing cider inoculated with *E. coli* O157:H7 at -20°C results in substantial cell death and injury; however, complete elimination is not achieved (Sage and Ingham, 1998). Survival in frozen ground beef for nine months (Doyle and Schoeni, 1987) and peptone water for seven months (Semanchek and Golden, 1998) has also been documented. Despite its ability to survive under such adverse conditions, the organism has no unusual resistance to heat. Splittstoesser et al. (1996) reported D-values of 12 minutes and one minute in cider when heated at temperatures of 52° and 58° C respectively. Semanchek and Golden (1996) also found fermentation to be an effective method for eliminating *E. coli* O157:H7 in apple cider. However, both heating and alcoholic fermentation cause changes in the characteristics of apple cider resulting in a different product altogether.

6. Pasteurization

Many believe that pasteurization is the best means of eliminating *E. coli* O157:H7 from apple cider. As mentioned above, the organism is easily eliminated by heat processing. Many larger juice processors have already begun using a pasteurization procedure in one form or another (Aylsworth, 1997). However, pasteurization may be

cost prohibitive for smaller operations as costs tend to increase sharply as production capacity and number of days per year of processing decrease (Kozempel et al., 1998). In addition, pasteurization may adversely affect sensory characteristics responsible for the appeal of fresh cider (Parish, 1997). Mandatory pasteurization would assure the safety of cider but is an unpopular choice among producers due to its costs and concerns about its effects on quality (Mclellan and Splittstoesser, 1996).

Due to the outbreaks and concerns about the safety of unpasteurized apple cider, the FDA has proposed a regulation requiring fruit and vegetable juice processors to implement HACCP programs. The regulation does not include mandatory pasteurization. However, it would require processors to adapt their processes to achieve a five log reduction in the number of pathogens in the finished product, the same level of reduction achievable through pasteurization (FDA, 1997).

7. Preventive Measures

HACCP has been defined as "a systematic approach to the identification, evaluation, and control of food safety hazards" and its underlying principal is prevention (NACMCF, 1998). Use of an effective HACCP plan can help to assure the safety of foods by incorporating several key measures that focus on the prevention of contamination (Mortimore and Wallace, 1995). An additional benefit is that it could also lead to better product quality (Parish, 1997). Prevention of contamination can begin in the orchard and continue through all steps involved in production.

Several practices involved in orchard management have been implicated in leading to contamination by *E. coli* O157:H7. Since cattle, deer, and other wildlife are carriers for *E. coli* O157:H7, contamination likely originates with contact or cross contamination from animal manure (Borczyk et al., 1987; Keene et al., 1997). Thus, manure should not be used to fertilize orchards (CFIA, 1998). Fencing could be used to help restrict the entry of animals into orchards (Mclellan and Splittstoesser, 1996). In addition, the use of drop apples for cider production is not advised since they may come in contact with manure. Fruit that has come into contact with the ground should be limited to use in products that receive a heat treatment (Parish, 1997).

Another potential source of contamination in the orchard is irrigation water; therefore, only microbiologically suitable water should be used (CFIA, 1998). It is also important for field workers to practice good personal hygiene and for harvesting and transport equipment to be clean (The Pennsylvania State University, 1998).

Research has shown that an overall tendency toward increasing populations of coliforms and aciduric microorganisms often exists as production progresses from growing to the final stages of cider production (Goverd et al., 1979; Swanson et al., 1985). If apples do become contaminated, other measures can be taken inside the processing facility to help assure that contaminates do not get carried over into the finished product. Procedures such as sorting, washing, and the use of sanitizers have already been covered in a previous section.

The threat of contamination from flume water is often great. Samples from the

flume waters of several producers all yield high coliform counts, possibly due to the practice of recycling flume water (Goverd et al., 1979). As with irrigation water, water used for fluming, washing, and rinsing should be potable and regular testing should be conducted to monitor microbiological quality (CFIA, 1998).

All facilities and processing equipment used in the manufacture of unpasteurized cider should be thoroughly cleaned and sanitized daily and treated in accordance with GMP's (FDA, 1996). Improperly cleaned and sanitized equipment can hold residual fruit and juice and may lead to the survival of aciduric microorganisms (Downing, 1989). It is also important to prevent outside sources of contamination, such as birds, insects, rodents, and small animals from gaining entry to the processing plant (The Pennsylvania State University, 1998). Workers also present a source of contamination and thus, good hygiene should be practiced by all plant personnel (Parish, 1997).

Refrigerated storage should be used for both apples and finished cider. While refrigeration is not lethal to *E. coli* O157:H7, it can reduce microbial growth and reproduction and slow spoilage and rotting which could lead to increased levels of contamination on apples (Parish, 1997). Refrigeration of cider is necessary to prevent fermentation and spoilage. However, refrigeration will not reduce or eliminate *E. coli* O157:H7. The survival of *E. coli* O157:H7 for up to 31 days in cider stored at 4-8°C has been documented (Zhao et al., 1993). In fact, researchers have documented enhanced survival in cider at 4°C when compared with survival at 25°C, especially at lower pH values (Miller and Kaspar, 1994).

While not required, routine laboratory testing could be conducted as part of a HACCP program. Sampling and testing at various points throughout the process could be used to help identify problems and to provide verification of product quality (The Pennsylvania State University, 1998). However, microbiological testing can not be relied upon to ensure safety. Some limitations of testing include the impracticality of 100% testing, the heterogenous distribution of microorganisms in the product, and the variability of analytical methods in sensitivity, specificity, and reliability (Mortimore and Wallace, 1994).

Labeling should also be considered. The use of lot/code numbers along with good record keeping can help to facilitate product tracking and recall, should a contamination problem arise (CFIA, 1998). In addition, FDA regulations now require warning labels on all packaged unpasteurized juice products (FDA, 1997).

8. Alternatives to Pasteurization

The FDA requirement for a five log reduction in pathogens present in the finished product would allow producers to employ means other than pasteurization to achieve the reduction. These measures may include, but are not limited to, washing, scrubbing, antimicrobial solutions, alternative technologies, or a combination of techniques (FDA, 1997). Some of the alternative technologies currently being investigated are irradiation, flash pasteurization, pulsed light, pulsed electric field, sterile filtration, microwave pasteurization, and ultraviolet light sterilization (Great Lakes Fruit Growers News, 1997;

Parish, 1997). Since this study deals with the use of antimicrobial solutions and UV light for reduction of *E. coli* O157:H7, only these alternatives will be discussed in detail. Washing, scrubbing, and the use of antimicrobial solutions have already been covered in a previous section. Ultraviolet light sterilization will be covered in greater detail in section D.

D. Ultraviolet (UV) Radiation

1. General

One of the physical methods available for sterilization is UV radiation. Although the UV region of the spectrum includes wavelengths between 15 and 450 nm, the region of interest with respect to antimicrobial activity lies between 220 and 300 nm (Block, 1977). Within this range, the wavelengths around 260 nm are the most effective for the destruction of microorganisms, since at this wavelength, absorption by nucleic acids is maximized (ICMSF, 1980).

When UV light energy is absorbed by nucleic acids, it causes mutations that can lead to cell death or cause sublethal injury (Jay, 1996). UV inactivation of microorganisms depends on both the intensity of UV energy and the duration of exposure (Block, 1977). The actual dosage in mw sec/cm^2 necessary to realize a certain level of inactivation can be expressed as the product of applied intensity (mw/cm^2) and irradiation time (Morgan, 1989). The resistance of a particular microorganism is often described in terms of the dose necessary to cause a 90% reduction in number (ICMSF,

1980).

2. Susceptibility of Microorganisms

In general, viruses and molds are more resistant to UV than bacteria (Farkas, 1997). The sensitivity of bacteria varies between different species and also depends on growth stage and the presence of spores (Block, 1977). Also, certain pigment forming bacteria are more resistant than those that form colorless colonies (Farkas, 1997). In addition, variations can exist between different strains of a particular species (ICMSF, 1980). In general, gram-negative rods are the most susceptible to UV radiation with gram-positive bacteria requiring about five times the dosage and bacterial spores about 10 times more (ICMSF, 1980). The dosage necessary to cause a reduction of 99.9% in *E. coli* is comparable to that required for many other vegetative bacteria (Chang et al., 1985).

3. Factors That Affect the Efficiency and Applicability of UV Sterilization

UV radiation has limited ability to penetrate substances and acts only at the surface, except for materials that are transparent to UV. In addition, sterilization can only occur within the direct radiation beam (Bachmann, 1975). Thus, the nature of the media in which microorganisms are suspended can greatly affect the effectiveness of UV light for their destruction.

UV radiation is unable to penetrate solid foods and has only limited ability to

penetrate liquids (ICMSF, 1980). Indeed, microorganisms are far more susceptible to the effects of UV when suspended in air rather than water (Block, 1977). The presence of small amounts of solutes or particulates in a liquid can greatly reduce UV penetration (Bachmann, 1975; Shama et al., 1996). According to Block (1977), the transmission of UV through liquids is inversely related to the mineral and organic content of the liquid. Organic particulate matter includes microorganisms and thus, high levels of microorganisms in liquids can also reduce the transmissivity of UV (Shama et al., 1996).

4. Applications

UV light has been used for some time for water sterilization and has shown effectiveness against a wide variety of microorganisms (Bachmann, 1975; Yip and Konasewich, 1972). It has been used for decontamination of air and surfaces in hospitals, laboratories, and food plants (Collins, 1971; ICMSF, 1980). UV radiation has also been used alone and with hydrogen peroxide for the treatment of packaging materials and for aseptic packaging (Farkas, 1997).

Direct exposure of foods and beverages themselves to UV light has been much more limited commercially. Spores of thermophilic bacteria in sugar crystals can be destroyed by application of UV light (ICMSF, 1980). Applied to meat surfaces in chill rooms, it has helped to delay spoilage (Block, 1977). In breweries, UV has been used as a final disinfection for deaerated liquor that is stripped of chlorine and thus, is susceptible to infection by spoilage organisms (Morgan, 1989).

Much research has been devoted to the direct application of UV light to food products. It has been used successfully on beef (Kaess and Weidermann, 1973; Stermer et al., 1987), fish (Huang and Toledo, 1982), and poultry (Sumner et al., 1996; Wallner-Pendleton et al., 1994) to control the growth of bacteria and increase shelf-life while causing little effect on food quality. However, UV treatment can have detrimental effects on certain foods. For example, it can accelerate the development of rancidity in foods with high levels of unsaturated fats and can cause discoloration of green leafy vegetables (ICMSF, 1980).

5. Treatment of Apple Cider

Little research exists on the use of UV to control pathogens in apple cider. It has been used successfully to lengthen the shelf-life of cider without affecting the flavor (USDA, 1977). However, commercial use has not been realized due to the suspended solids in cider (Downing, 1989). Kissinger and Willits (1966) used it to eliminate microorganisms in flowing maple sap. However, the dissolved solid content of sap is much lower (1-4%) than that of apple juice (9.8-16.9%) (Mattick and Moyer, 1983).

For UV radiation to be effective in such liquids, the liquid must be subjected to UV in the form of a thin film (Downing, 1989). In this manner, the absorption of UV by the liquid itself is minimized allowing for greater absorption by microorganisms (Shama, 1992). The inactivation of *E. coli* in water and humic acid solutions of high UV absorptivities with a thin film UV apparatus has been documented. In this study, the

fractional survival of *E. coli* in water with an absorption coefficient of 0.18 was 1.88 x 10^{-5} and that in humic acid solution with an absorption coefficient of 4.0 was 1.84 x 10^{-4} following UV treatment (Shama, 1992).

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SECTION II: REDUCTION OF *ESCHERICHIA COLI* O157:H7 ON APPLES USING WASH AND CHEMICAL SANITIZER TREATMENTS

ABSTRACT

Unpasteurized apple cider has been implicated in outbreaks involving *Escherichia coli* O157:H7. Apples used for cider production may become contaminated by contact with animal feces. The objective of this study was to determine if wash and sanitizer treatments can reduce or eliminate *E. coli* O157:H7 on apples. Apples were subjected to six wash or sanitizing treatments including 200 ppm hypochlorite, a commercial phosphoric acid fruit wash, 5% acetic acid, 5% acetic acid followed by 3% hydrogen peroxide, a commercial peroxyacetic acid sanitizer, and distilled water. Apples inoculated with a five-strain mixture (~2 x 10^3 CFU *E. coli* O157:H7 per cm^2) were immersed in treatments for two minutes. The water wash caused reductions of only 1.1 logs when cells were enumerated on Sorbitol MacConkey agar (SMAC) and 0.6 logs using Tryptone Soy agar with 1% pyruvic acid (TSAP) and was the only treatment that did not differ significantly from the no wash control. Hypochlorite caused reductions of 2.1 logs on both media but differed significantly from the most effective treatment, 5% acetic acid. Phosphoric acid resulted in a reduction of 2.9 logs when cells were enumerated on SMAC but only 2.3 logs using TSAP as the recovery medium indicating that the treatment caused some sublethal injury. For the acetic acid/hydrogen peroxide treatment, there was a 2.5 log reduction using SMAC and 2.4 logs with TSAP. The 5%

acetic acid and peroxyacetic acid solutions were the most effective with reductions of 3.1 logs and 2.6 logs respectively, without apparent sublethal injury.

INTRODUCTION

Escherichia coli O157:H7 was first identified as a foodborne pathogen in 1982 and is now acknowledged as a significant cause of foodborne illness (*15*). Illness due to *E. coli* O157:H7 can range from self-limited, watery diarrhea to the more severe hemorrhagic colitis, hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP) (*28*).

One of the earliest outbreaks of HUS associated with apple juice or cider occurred in Canada two years before the recognition of *E. coli* O157:H7 as a foodborne pathogen; however, the agent responsible was not identified, possibly due to the length of time between sampling and analysis (*33*). Outbreaks of HUS in Massachusetts in 1991 (*4*) and Connecticut in 1996 (*10*) attributed to *E. coli* O157:H7 were associated with the consumption of contaminated apple cider. In a 1996 outbreak involving unpasteurized apple juice in the pacific northwest, Odwalla brand apple juice and juice mixtures contaminated with *E. coli* O157:H7 were implicated and caused a nationwide recall of all products containing apple juice (*9*).

Although the specific mechanism of contamination of apple cider with *E. coli* O157:H7 is often unknown, several explanations have been offered. Since cattle and other ruminants are generally regarded as the primary reservoir for this organism (*6*), contamination most likely originates directly or indirectly from fecal matter. Direct contamination may result from the use of fallen apples, fertilization of orchards with manure, or even grazing of farm animals in close proximity to orchards. Other

possibilities include poor hygiene and unsanitary procedures of field and processing staff, inadequate cleaning of processing equipment, the use of decayed or damaged fruit, and failure to wash apples properly before processing (*4, 35*).

Many believe that the use of a kill step such as pasteurization rather than prevention of contamination is the best means of eliminating *E. coli* O157:H7 from apple cider. Some of the larger juice processors have already begun using a pasteurization procedure (*2*). However, pasteurization may be cost prohibitive for many smaller operations as costs increase sharply as production capacity and number of days per year of processing decrease (*22*). In addition, pasteurization may have adverse effects on the sensory characteristics responsible for the appeal of fresh cider (*29*).

Besides pasteurization, or another means of sterilizing the final product, the preventive measure that may prove to have the greatest impact on the microbiological safety of cider is the use of a wash and/or sanitizing treatment on apples before processing. Such a measure may be the easiest to implement and monitor and may result in only a moderate increase in the cost of production. A typical wash procedure employs water or chlorinated water, may be used with or without a scrubber, and is designed for the removal of field soil prior to processing (*14*). In addition, a commercial fruit wash can be added to wash water to facilitate the removal of field soil. There is little research on the effectiveness of such products for the removal of bacteria. One such product was evaluated during this study.

Sanitizing compounds, such as a chlorine solution, used alone, or in conjunction

with a wash step, may also be employed (*8*). In fact, chlorine solutions at concentrations of 50-200 ppm are the most widely used treatments for fresh produce with a typical contact time of 1-2 minutes (*16*). Organic acids such as acetic acid have GRAS status and have been shown to have antimicrobial properties. Hydrogen peroxide is also known for its bactericidal effects and is rapidly decomposed leaving no residual toxicity (*12*). Peroxyacetic acid displays good antimicrobial activity against a wide variety of microorganisms (*12*).

The objective of this study was to evaluate the effectiveness of various wash and sanitizer treatments for the elimination of *E. coli* O157:H7 from the surface of apples.

MATERIALS AND METHODS

Preparation of inoculum

Five acid resistant *E. coli* O157:H7 strains 380-94, 933, C7927 (human isolate from cider outbreak), E0019, and E09 were obtained from the University of Nebraska, Lincoln culture collection. Stock cultures were maintained on Tryptone Soy agar (TSA) (Difco Laboratories, Detroit, MI) at 4^{o} C and grown in Tryptone Soy broth (TSB) (Difco Laboratories) at 35^{o} C. Preliminary research determined that the level of inoculum obtained in TSB for the five individual cultures was relatively even (8.0×10^{8} - 1.5×10^{9} CFU/ml). Each culture was subjected to two successive transfers by loop inocula to 10 ml TSB. A third transfer of 1 ml was made into 100 ml TSB adjusted to pH 5 with 1 N HCl. This step was included to allow the cultures to become acid adapted as reported by

Leyer et al. (*23*). Incubation of cultures was for 18-24 hours at 35°C. Cultures were then combined in equal volumes to create a five-strain mixture. A 30 ml aliquot from the mixed culture was added to 9 liters sterile distilled water at 25°C in a 7 gal polypropylene tank (Nalgene, Rochester, NY). Preliminary studies showed that this cell suspension routinely resulted in a level of approximately 1 x 10^6 *E. coli* O157:H7 per ml. This suspension served as the inoculum for the test apples.

Preparation and inoculation of apples

Sound, blemish free Red Delicious apples of uniform size and shape (2 ½ to 2 3/4 inches in diam) were obtained from the Virginia Tech fruit and vegetable processing plant and assigned randomly to treatments. Apples were allowed to adjust to room temperature (~25° C) before inoculation. Apples were placed in the inoculum and agitated by stirring with a glass rod for 10 min to ensure even inoculation. For each replication, a portion of the apples were not inoculated and served as controls to check for natural flora. Inoculated apples were allowed to dry for at least 30 min in a laminar flow biological hood before treatment.

Preparation of wash and sanitizer treatments

Five wash or sanitizer treatments were prepared; 200 ppm sodium hypochlorite, Decco APL Kleen® 246 (Elf Atochem North America, Inc., Monrovia, CA), a commercial phosphoric acid-based fruit wash (0.3% phosphoric acid), 5% acetic acid V/V, 5% acetic acid + 3% hydrogen peroxide, and Tsunami 100™ (Ecolab®, Food and Beverage Division, St. Paul, MN) a commercial peroxyacetic acid-based solution (80

ppm peroxyacetic acid). A distilled water treatment was also included as a control. Distilled water for all treatments was allowed to adjust to room temperature (~25 °C) before the addition of chemicals and introduction of apples. Treatments were prepared in 2 gal polypropylene tanks (Nalgene) and stirred to mix before application.

Application of wash and sanitizer treatments

Apples were placed in the treatment tanks and stirred with a glass rod to ensure even contact with the solution. Since a contact time of 2 minutes or less is generally employed with hypochlorite solutions used for produce (*16*), a 2 min contact time was employed for each treatment for the sake of comparison. Contact time was determined by a stopwatch. For the combination treatment of 5% acetic acid followed by 3% hydrogen peroxide, contact time was 1 min in each solution. For each apple, treatments were followed by a 10 ml distilled water rinse applied by a hand-held sprayer (Fisher Scientific, Pittsburgh, PA). Apples were allowed to dry in a laminar flow biological hood for at least 30 min before analysis. For each replication, a portion of the inoculated apples did not receive any treatment and served as a control to determine the actual inoculum level.

Analysis and enumeration

Following treatment, apples were placed individually in stomacher bags to which 100 ml of 0.1% sodium lauryl sulfate solution (SLS) (Fisher Scientific) was added. SLS was used to facilitate removal of any bacteria remaining on apple surfaces. Preliminary studies demonstrated that recovery was generally better with SLS solution than for 0.1%

peptone solution. Each bag was massaged by hand for 1 minute. Serial dilutions were made in 0.1% peptone (Difco Laboratories) and spread plated in duplicate on Sorbitol MacConkey agar (SMAC) (Difco Laboratories), and TSA supplemented with 1% pyruvic acid (Fisher Scientific) (TSAP). Recovery of injured *E. coli* O157:H7 is best using nonselective media such as TSA (*32*), thus the nonselective TSAP was used for the recovery of sublethally injured cells. Plates were incubated for 20-24 h at 35°C. Sorbitol negative colonies on SMAC and morphologically typical colonies on TSAP were enumerated with a Quebec colony counter. Routine verification of isolates was conducted using Micro-ID's (Remel, Lenexa, KS), and *E. coli* O157 Latex agglutination test kit (Unipath Oxoid USA).

Experimental design and statistical analysis

The experiment was replicated six times and multiple samples from each of the 7 treatments plus the uninoculated control were analyzed at each sampling time. The total number of samples for each treatment plated on SMAC and TSAP was 23 and 16 respectively. Microbial counts (CFU per square centimeter of apple surface area) were determined in duplicate for each replication. Counts were subjected to the Kruskal-Wallis Test and Fisher's Exact Test (2- Tail) using the Statistical Analysis System (SAS Institute, Cary, NC) to determine significant differences ($P \leq 0.001$ unless otherwise noted) among treatments.

RESULTS AND DISCUSSION

Wash and sanitizer treatments

E. coli O157:H7 was not detected on apples that were not inoculated and the level of aerobic mesophilic background microflora on apples was <10 CFU/cm^2. Preliminary studies were conducted to determine the level of inoculum that could be obtained on the surface of apples. Although the level of inoculum obtained for the test apple suspension was routinely >10^6 CFU/ml, the level achieved for apple surfaces ranged from 4.6 x 10^3 to 8.0 x 10^3 CFU/cm^2. This was also the case for the control treatment that did not receive a wash or sanitizing step. As indicated in Figures 1 and 2, the mean counts of *E. coli* O157:H7 for the no wash control were 1.4 x 10^3 CFU/cm^2 on SMAC and 2.7 x 10^3 CFU/cm^2 for TSAP, respectively.

Mean counts of *E. coli* O157:H7 on apple surfaces after treatments as recovered on SMAC are shown in Figure 1. As shown, all treatments except water and 200 ppm hypochlorite resulted in a reduction of 2.5 logs or greater. All treatments, except water, differed significantly from the inoculated control that did not receive a wash. Likewise, all other treatments were significantly different from water alone. Reduction of *E. coli* O157:H7 on apple surfaces from water alone was 1.1 logs. No significant difference between chemical treatments was seen except for that between 5% acetic acid and 200 ppm hypochlorite ($P \leq 0.022$). The reduction achieved with 200 ppm hypochlorite, adjusted to pH 5 to maximize the amount of free available chlorine in solution, was 2.1 logs. Mean counts on SMAC for chemically treated apples ranged from 1.17 CFU/cm^2 for 5% acetic acid to 11.91 CFU/cm^2 for 200 ppm hypochlorite.

Results obtained using the injury recovery media TSAP are shown in Figure 2. Overall, results were similar to those obtained with SMAC although some differences were evident. When allowing for the recovery of sublethally injured organisms, only two of the chemical treatments (5% acetic acid and peroxyacetic acid) resulted in a reduction of 2.5 logs or greater. All treatments including water were significantly different from uninoculated controls and all chemical treatments differed from water alone. As with SMAC, the only chemical treatments to differ significantly were 5% acetic acid and 200 ppm hypochlorite ($P \leq 0.018$). Mean counts on TSAP for chemically treated apples ranged from 1.25 CFU/cm^2 for 5% acetic acid to 23.28 CFU/cm^2 for 200 ppm hypochlorite. The reduction of approximately one log *E. coli* O157:H7 seen here when using the water dip treatment is consistent with that reported by other researchers. Brackett (*7*) reported a 1 log reduction of *Listeria monocytogenes* on Brussels sprouts after dipping in water. Likewise, Harmon et al. (*20*) found that washing mung bean sprouts 3 times by spraying with water for five minutes reduced *Bacillus cereus* by approximately 1 log. Nguyen and Carlin (*27*) saw bacterial reductions of less than one log when using water as a wash for many different vegetables. Also, water alone had a minimal effect on the microbial load of packaged salad mix (*31*).

Since water has no antimicrobial activity at the temperature at which it was used in this study, the reduction must have been due to bacteria simply being washed off the surface of the apples. There may be a limit to the amount of cells that can be removed in this way. A water wash used in conjunction with some physical means, such as

brushing, to better facilitate removal of bacterial cells may result in a greater reduction. In a survey of Virginia cider producers practices, 61% of respondents indicated that they use brushing along with washing (*36*). However, the results of this study clearly show that a water dip treatment may be inadequate for the removal of *E. coli* O157:H7 from apple surfaces. This may be particularly true for heavily soiled fruit. This finding appears even more important given that only 17% of those surveyed use any type of detergent wash and only 34% use a sanitizer. The majority of processors use water washing alone (*36*).

Cider producers often use chlorinated water from a municipal water supply to wash apples. Chlorine is also widely added to wash water in fruit and vegetable processing plants as a sanitizer (*5*). However, the antimicrobial activity of hypochlorite is reliant on environmental factors such as the pH, temperature, organic load, and ionic concentration of the solution (*12, 37*). Goverd et al. (*19*) investigated the frequency of coliforms and *Salmonella* in cider and juice processing plants and saw an ongoing and cumulative bacterial contamination from fruit production to finished product. Coliform counts of flume water ranged from $> 1.8 \times 10^3$ to 8.0×10^6. The microbial counts of fruits and vegetables and thus, the organic load in fruit and vegetable wash and flume water is often high (*27*). In addition, wash solutions are often recycled and this leads to a higher organic load and a greater chance of contamination of fruit (*8*). Garg et al. (*17*) found that maintaining the desired level of free available chlorine in wash solutions in the processing plant was difficult. This was attributed to organic material in solution

from vegetables and to the fact that chlorine addition was done manually.

For this study, a 200 ppm hypochlorite solution, adjusted to pH 5 to maximize available chlorine, was used. At a pH of 4-5, 98-100% of the chlorine is in the hypochlorous acid (HOCl) form or that which has the greatest bactericidal activity (*12*). Wash water was not recycled, the apples were not highly soiled, and the level of background microflora was low. Under such conditions, the effectiveness of chlorine is maximized as has been shown by other researchers in laboratory studies (*1*). In this study, the mean count of *E. coli* O157:H7 on apple surfaces after chlorine treatment was 11.91 CFU/cm^2 on SMAC and 23.28 CFU/cm^2 on TSAP. These were the highest counts seen among the chemical treatments. Although these numbers may seem low, the infective dose of *E. coli* O157:H7 is believed to be very low (*25*). This study was done under controlled conditions and the effectiveness of hypochlorite in the processing plant may be reduced as mentioned above. Several researchers have reported less than ideal results when using a hypochlorite solution as an antimicrobial wash treatment for various fruits and vegetables (*1, 7, 18, 34, 37, 38*). Some possible reasons given for the ineffectiveness of hypochlorite solutions in these studies are inactivation of hypochlorite by organic matter, incomplete wetting of produce (*7*), and the inaccessibility of chlorine to crevices and pockets (*1*).

The hypochlorite treatment was the only chemical treatment found to differ significantly from any of the other chemical treatments. This difference was seen between hypochlorite and 5% acetic acid, which resulted in the lowest mean counts on

both media. Only small differences were seen between the other chemical treatments and all are more effective for the elimination of *E. coli* O157:H7 from apples than either water or hypochlorite solution.

The phosphoric acid-based fruit wash resulted in the second largest reduction (2.9 logs) when plating on SMAC. However, it was better than only hypochlorite when using the injury recovery media and resulted in a reduction of 2.3 logs. This suggests that the phosphoric acid may have caused sublethal injury to a portion of the bacterial cells. However, the difference between SMAC and TSAP was not statistically significant. It should be noted that this product is described by the manufacturer as a general purpose acidic cleaner and is not intended to be a sanitizer. It is designed for the removal of field soil from apples and pears.

Hydrogen peroxide used in chiller water for poultry at a concentration of 6600 ppm is an effective bactericide but it leads to bleaching and bloating of carcasses (*24*). Researchers investigating the effectiveness of several sanitizers for the removal of *E. coli* O157:H7 from produce, saw a reduction of 2 logs on broccoli and 4 logs on tomatoes using 3% hydrogen peroxide. However, the most effective treatment, 5% acetic acid followed by 3% hydrogen peroxide, reduced the organism to undetectable levels (*30*). This treatment is also an effective means of reducing the bacterial load on beef carcass tissue (*3*). We chose to include this treatment in our study based on the results of these earlier works. In this study, the combination treatment of acetic acid/hydrogen peroxide caused a reduction of 2.5 logs when cells were enumerated on SMAC and 2.4 logs using

TSAP. This treatment was slightly less effective than acetic acid alone. Increasing the contact time in each solution may result in a greater reduction. However, as mentioned previously, these treatments did not differ significantly.

Little research exists on the use of peroxyacetic acid for the removal of pathogens from the surfaces of produce. It is increasingly used in clean-in-place sanitizing in beverage and dairy plants due to its effectiveness against yeasts and molds (*26*). It has also been used as a sanitizer for food contact surfaces and has been found to be effective for the inactivation of various pathogens including *Listeria monocytogenes*, *Yersinia enterocolitica*, and *Campylobacter jejuni* (*12*). In the present study, the peroxyacetic acid treatment, like acetic acid, tended to kill the organism rather than cause injury, though reduction was slightly lower than that for acetic acid.

Studies involving the antimicrobial effectiveness of acetic acid have seen mixed success. Zhang and Farber (*37*) saw *Listeria monocytogenes* reductions of less than one log on lettuce and cabbage treated by dipping in 1% acetic acid. More concentrated solutions of acetic acid were not tested; however, as higher concentrations may have had adverse effects on the quality of these leafy vegetables. Organic acid spray treatments including acetic acid, in concentrations up to 5%, were used with some success to reduce *E. coli* O157:H7 on beef tissue; however, total elimination is not achieved (*11*). Karapinar and Aktug Gonul (*21*) achieved a 5 log reduction of *Yersinia enterocolitica* on parsley by dipping in both 2% and 5% acetic acid for 15 minutes. *E. coli* O157:H7 is reduced by 2 logs on broccoli and 3 logs on tomatoes with little effect on quality when

acetic acid solutions of 2% and 5% are used (*30*). In this study, 5% acetic acid was the most effective treatment for the elimination of *E. coli* O157:H7 from the surface of apples resulting in a reduction of 3.1 logs without causing sublethal injury.

Based on the results of this study, it appears that the use of a sanitizing treatment in conjunction with a water or commercially available detergent fruit wash for cider apples may help to reduce *E. coli* O157:H7 in apple cider. The purpose of the wash step is to remove field soil and it should not be relied upon for the removal of pathogenic microorganisms. Any of the chemical solutions used in this study, with the exception of hypochlorite, could be used as a sanitizing treatment following the wash step and should be more effective for the reduction of *E. coli* O157:H7 than practices currently used by the majority of producers. Both 5% acetic acid and 80 ppm peroxyacetic acid were effective for the reduction of *E. coli* O157:H7 from apple surfaces without causing sublethal injury. More research is needed however, to determine the effectiveness of such treatments under actual processing conditions.

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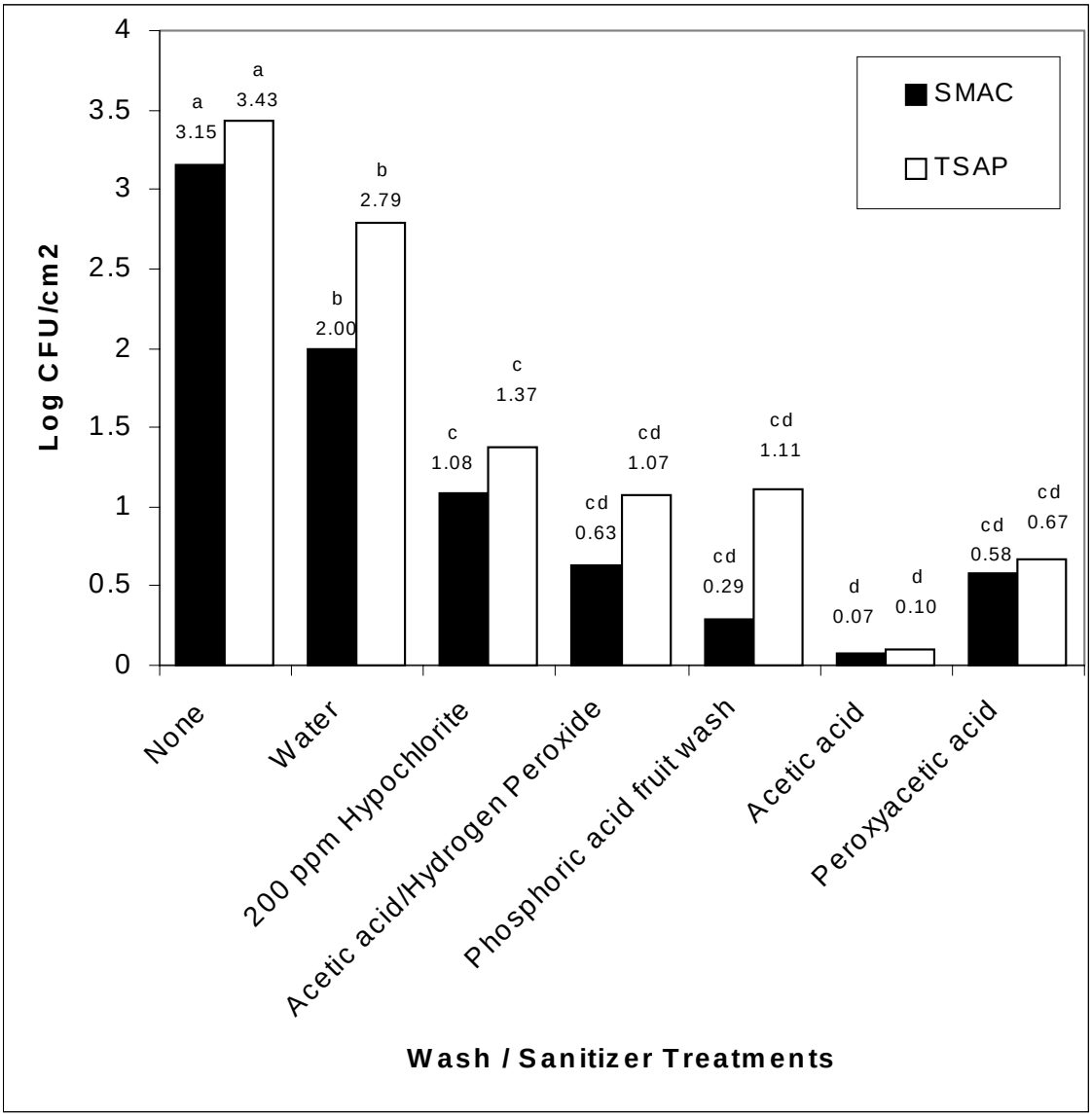

Figure 1.Populations of *E. coli* O157:H7 on apples subjected to wash or sanitizer treatments as enumerated on Sorbitol MacConkey agar (SMAC) and Tryptone soy agar with 1% pyruvic acid (TSAP).

SECTION III: A SURVEY OF VIRGINIA CIDER PRODUCERS PRACTICES

ABSTRACT

During the summer of 1998, Virginia cider producers were surveyed to gain a better understanding of current production practices. The survey included questions covering production levels and sales location, orchard management, facilities, processing, preservation measures, and additional safety measures. Most operations are seasonal, produce less than 5,000 gal annually, and confine sales to Virginia. Although few producers fertilize orchards with manure, animals often graze nearby. Also, drop and damaged apples are sometimes used. Most process in separate, enclosed areas and test water for bacteria. All indicate that equipment and facilities are cleaned and sanitized daily. The majority of producers sort apples before washing, use refrigerated storage, prevent contamination during storage, and wash and brush apples before pressing. However, most use only water for washing and only 37% use a chemical sanitizer following the wash step. Few pasteurize cider or add preservatives; however, almost all store cider refrigerated or frozen. Although only 25% have an operating HACCP program, most have considered implementing HACCP. Few producers conduct microbiological tests on cider or include code numbers or expiration dates on labels. Finally, most producers expressed an interest in the use of alternative processing technologies to help assure the safety of their cider.

INTRODUCTION

The 1997 apple crop in the United States was valued at over 1.5 billion dollars, giving some indication of the importance of apples as an agricultural product (*37*). Apples rank as the third most significant fruit crop in terms of annual production and consumption of apple juice ranks second to orange juice among fruit juices in the U.S. (*12*). Approximately 21% of the 1997 apple crop was processed into juices including apple cider (*37*).

Prior to the 1940's, most apple juice was consumed fresh in the form of cider and was mainly a seasonal beverage (*12*). Since that time, cider production has decreased due, in part, to the rapid growth of commercially canned juices (*38*). However, apple cider has its own distinctive appeal, is desired by many consumers, and provides an important source of income for apple growers (*38*). In the U.S., there are approximately 9,000 apple growers with orchards covering over 459,000 acres (*37*).

Recently, outbreaks of hemolytic uremic syndrome (HUS) involving *Escherichia coli* O157:H7 in unpasteurized apple cider and juice have generated concern about its safety (*1,8,9*). While apple juice is often pasteurized, cider producers have relied upon the products inherent acidity as well as refrigeration, and chemical preservatives for preservation (*12*). However, research has shown the ability of *E. coli* O157:H7 to survive in cider despite its low pH and regardless of whether refrigeration or preservatives are used (*28,42*).

Therefore, alternative means of assuring the safety of unpasteurized cider are

currently being sought. The purpose of this study was to gain a better understanding of practices currently used in the production of apple cider and of how research may be used by the cider industry to help assure the production of a safe and quality product.

THE SURVEY

During the summer of 1998, a survey of cider producer practices was developed and sent to cider producers in Virginia. A list of names and addresses of producers was obtained from Virginia Apple Growers Association (Richmond, VA). The survey consisted of 30 questions pertaining to cider production and included a request for comments. Questions dealt with production levels, orchard management, facilities, fruit handling and processing, preservation measures, and additional safety measures. The survey was conducted on a basis of anonymity to assure the best possible response rate.

A total of 42 surveys were returned and the total number of valid responses for specific questions ranged from 38 to 42. Some respondents did not provide answers for all questions. Three of the questions either did not apply to all producers or were dependent on prior questions and thus, had fewer responses.

PRODUCTION LEVELS AND LOCATION OF SALES

It appears that a large portion of the Virginia cider industry consists of small, seasonal operations that market their product locally. As shown in Table 1, the majority (59%) of respondents produce <5,000 gal of cider per year and only 12% produce

>50,000 gal annually. A survey of Michigan cider producers revealed similar findings with the majority of operations producing <5,000 gal and very few producing >100,000 gal (*40*). Most processors (79%) sell only in Virginia and grow all of their own apples (67%). In addition, in the comments section, several respondents described their operations as small and seasonal. Seasonal production and in state sales were also characteristic of Michigan producers (*40*). Virginia producers also expressed concerns about new regulations and a few stated that they would likely cease production if faced with additional expenses.

ORCHARD MANAGEMENT

Responses to questions relating to orchard management are given in Table 2. Only 8% of processors use manure to fertilize orchards and only 5% indicate that domesticated animals are permitted to graze in orchards. These findings are in agreement with those of the Michigan survey (*40*). However, 54% of respondents noted that animals do graze in adjacent fields. In addition, 32% of producers reported using drop apples. The majority (66.6%) of those who use drops gather drop apples at least twice per week; however, 25% gather drops only at the time of picking. In comparison, the Michigan survey reported that the use of drops was considerable and that drop apples were allowed to remain on the ground for some time prior to gathering (*40*). In a survey of cider producers attending a meeting of the New England Fruit Growers conducted by the CDC, all 36 respondents reported that they use drop apples (1).

Since cattle, deer, and other wildlife are carriers for *E. coli* O157:H7, contamination is likely due to contact with or cross contamination from animal manure (*4,23*). Consequently, the use of drop apples for cider production is a risky practice. Fruit that has come into contact with the ground should be limited to use in products that receive a heat treatment to reduce the possibility of *E. coli* O157:H7 contamination (*31*). Fencing could be used to help restrict the entry of animals into orchards (*26*).

FACILITIES

Survey questions dealing with facilities are depicted in Table 3. Seventy-nine percent of respondents carry out all processing steps in a separate, enclosed room or building and all reported that facilities and processing equipment are cleaned and sanitized at the end of production each day. Such practices are important to prevent outside sources of contamination, such as insects and rodents, from gaining entry to the processing plant and to prevent cross contamination from processing equipment (*36*). In an investigation of the prevalence of acid and heat resistant bacteria in apple cider and juice plants, it was found that bacterial counts increased gradually during daily production. The increase was attributed to microbial growth in or on production equipment (*35*). Improperly cleaned and sanitized equipment results in residual fruit and juice and may lead to the survival of aciduric microorganisms (*12*).

The microbiological quality of cleaning water is very important and coliform testing is required for most water supplies used for food processing (*17*). Most producers

(72.5%) noted that their water supplies are tested regularly for coliform bacteria. Wash and flume water should be tested regularly to ensure that it does not become a source of contamination (*6,18*). *E. coli* O157:H7 can survive for long periods in water, especially cold water (*39*). In a study of cider and juice processing facilities, it was found that coliforms increased in wash and flume waters during the production day (*19*). This finding provides additional impetus for starting with clean water that is free of coliform bacteria.

FRUIT HANDLING AND PROCESSING

Responses to questions related to fruit handling and processing are given in Table 4. Almost all (98%) processors sort apples to remove wormy, rotten, or otherwise unacceptable fruit and 82% of those who do, sort apples before washing. However, 37.5% acknowledge that damaged fruit is used for cider production. These results are consistent with those of the Michigan survey (40). The removal of damaged fruit prior to washing is advised since it may harbor bacteria that could be spread through wash water to sound apples (*3*). The waxy skin of apples provides a barrier to microbial entry, however, breaks in the skin weaken this natural defense (*11*).

Most (63%) producers refrigerate apples that are not used immediately and 80% indicate they take measures to prevent additional contamination during storage. While refrigeration is not lethal to *E. coli* O157:H7, it can reduce microbial growth and reproduction and slow spoilage and rotting which could lead to increased levels of

contamination (*31*). The prevention of contamination during storage is also critical and such measures will help to maintain the microbiological quality of the fruit. Insects, birds, small animals, and other pests are potential sources of coliform bacteria and should be excluded from storage areas and all parts of the processing facility (*16*).

Produce is cleaned to remove field soil, pesticide residues, insects, microorganisms, and other extraneous matter prior to processing (*18*). A typical wash procedure uses water or chlorinated water, may include scrubbing, and is intended for the removal of field soil prior to processing (*12*). Most processors (93%) use a wash procedure to clean fruit prior to pressing and 64% also use brushing. However, most use only water to wash apples. Again, this is consistent with the findings of the Michigan survey (40); however, only 33% of the New England respondents reported that they always wash apples before pressing (1). This disparity may be due to the timing of the New England survey, which was conducted in 1991 before most of the larger outbreaks had occurred.

Although a water wash may be adequate for the removal of field soil, it often has little effect on surface bacteria. Several researchers have demonstrated the inability of water washes to remove bacteria from the surface of fruits and vegetables (*5,21,30,34,41*). Only 18% of processors indicated that they use a detergent to facilitate soil removal. The addition of an approved detergent or a commercially available fruit wash to wash water may help to remove field soil and associated microorganisms from apple surfaces (*27*). A commercial phosphoric acid-based fruit wash significantly

reduced *E. coli* O157:H7 on apple surfaces when applied as a dip treatment (*41*).

Although the importance of washing should not be underestimated, it is equally important to consider a sanitizing treatment for fruit used in the production of unpasteurized juices (*31*). An important distinction should be made between cleaning and sanitizing. Cleaning can be defined as the physical removal of soil while sanitizing consists of chemical or heat treatment designed for the removal of microorganisms (*25*).

The ineffectiveness of water washing is even more troubling given that only 37% of respondents use any type of sanitizing treatment for apples following the wash step. The use of detergents and sanitizers is not common among Michigan cider producers either (40). Water from municipal supplies is often chlorinated and chlorine is widely added to wash water in fruit and vegetable processing plants (*2*). However, the antimicrobial activity of chlorine is reliant on environmental factors such as the pH, temperature, organic load, and ionic concentration of the solution (*10*). The microbial counts of fruits and vegetables and thus, the organic load in wash and flume water is often rather high (*30*). In addition, wash solution is often recycled which leads to a high organic load and a greater chance of contamination (*6*). Also, it has been shown that maintaining the desired level of free available chlorine in wash solutions is often difficult due to high levels of organic material in solution, especially if chlorine is added manually (*15*).

Due to the ineffectiveness of water and chlorine solutions, producers of unpasteurized juices may want to consider using other chemical sanitizer treatments.

Organic acids such as acetic acid have GRAS status and have been used successfully to remove pathogens from produce (*22,32*). In a comparison of several treatments, acetic acid at a concentration of 5% was the most effective treatment for the removal of *E. coli* O157:H7 from the surface of apples (*41*). Hydrogen peroxide is also known for its bactericidal effects and is rapidly decomposed leaving no residual toxicity (*10*). Hydrogen peroxide at a concentration of 3% proved to be effective for removing *E. coli* O157:H7 from the surface of tomatoes. Even greater effectiveness was seen when hydrogen peroxide was used in combination with acetic acid (*32*).

PRESERVATION MEASURES

Table 5 details preservation measures taken with the finished product. Most (78%) producers do not pasteurize their cider. This is not surprising given the small size and seasonal nature of most Virginia operations. Pasteurization may be cost prohibitive for such operations since the costs associated with it increase sharply as production capacity and number of processing days per year decrease (*24*). Also, a heat treatment may adversely affect the sensory characteristics responsible for the appeal of fresh cider (*31*). Pasteurization is also not frequently used by Michigan producers (40).

Just 12% of producers add preservatives to cider. Of those who do, 60% use potassium sorbate and 40% use sodium benzoate. In laboratory studies, potassium sorbate had little effect on *E. coli* O157:H7 populations in cider (*28,42*). Likewise, *E. coli* O157:H7 survived in refrigerated cider containing 0.1% sodium benzoate for 21

days (*28*). Thus, while preservatives have merit for the extension of shelf-life, they cannot be relied upon to eliminate *E. coli* O157:H7 from apple cider. According to the New England survey, the use of preservatives is also not common among producers in that region (1).

The most popular preservation measures are refrigeration and freezing. Almost all (93%) producers chill cider immediately after production and 92.5% either refrigerate cider or freeze it until sale. Prompt cooling and subsequent refrigeration of unpasteurized cider is necessary to retain the best flavor and to prevent fermentation. Freezing can extend the shelf life of cider for even longer periods (*12*). However, neither are reliable measures for the elimination of pathogens. The survival of *E. coli* O157:H7 for up to 31 days in cider stored at 4-8^{o} C has been documented (*42*). Survival in ground beef for 9 months (*13*) and peptone water for 7 months (*33*) when stored at - 20^{o}C has also been shown.

ADDITIONAL SAFETY MEASURES

The final area of investigation focused on additional measures that can be taken to help assure the safety of apple cider and responses to these questions are shown in Table 6. Such measures include microbiological testing, labeling, HACCP, and alternative technologies. While only 25% of cider producers indicated they have an operating HACCP program, 67% of those who don't have a HACCP program have considered implementing one. This interest in HACCP may be due in part to the recent

proposal by the FDA for a regulation to require processors of unpasteurized juices to implement HACCP programs. The regulation also includes a requirement for processors to adapt their processes to achieve a five log reduction in the number of pathogens present in the final product (*14*). Use of an approved HACCP plan may help to assure the safety of unpasteurized cider by incorporating several key measures that focus on the prevention of contamination (*29*). An additional benefit of HACCP is that it could also lead to the production of a higher quality product (*31*).

Very few producers (2%) conduct microbiological testing on cider and this is consistent with the Michigan survey (40). While not required, laboratory testing could be instituted as part of a HACCP program for verification purposes. Sampling and testing at various points throughout the process could be used to help identify problems and to provide confirmation of product quality (*36*).

Only 2% of cider producers label their product with an expiration date and just 14% include an identifying lot or code number. In contrast, approximately 50% of respondents to the Michigan survey include expiration dates; however, most do not use lot codes (40). An expiration or freshness date might be included to encourage consumption of the product while at peak quality. Labeling can also be used to provide handling instructions (*36*). The use of lot/code numbers along with good record keeping can help to facilitate product tracking and recall, should a contamination problem arise (*7*).

Finally, 80% of Virginia cider producers said they would be interested in using

alternative processing technologies to help assure the safety of their products. The requirement by the FDA for a five log reduction in the numbers of pathogens present in the finished product would allow producers to employ means other than pasteurization to achieve the reduction. These measures may include washing, scrubbing, antimicrobial solutions, alternative technologies, or a combination of techniques (*14*). Some alternative technologies currently being investigated are irradiation, flash pasteurization, pulsed light, microwave pasteurization, and ultraviolet light sterilization (*20*).

CONCLUSIONS

Although most of the Virginia cider operations are small, seasonal businesses, their importance to the economy and to Virginia agriculture should not be underestimated. As one respondent commented, "Cider making is a process to help apple producers to stay in the apple business." However, the serious nature of the recent outbreaks of *E. coli* O157:H7 linked to the consumption of unpasteurized cider underscores the fact that the safety of the product as currently produced cannot be overlooked.

Mandatory pasteurization would assure the safety of cider; however, it is an unpopular choice among producers due to its costs and concerns about its effects on quality. Indeed, very few of those surveyed pasteurize their cider and some said that such a measure would be cost prohibitive and likely cause them to cease production if required. The FDA regulations for HACCP may seem intimidating to some producers.

However, our survey suggests that many of the methods mentioned by the FDA that could be employed to achieve a five log reduction are already being used to some degree by many of the Virginia processors.

Most producers appear to be using sound orchard management practices with the exception of the use of drop apples and damaged fruit. The discontinuation of such practices would help assure that only the highest quality raw materials are used for the production of unpasteurized cider. Likewise, most producers already use a wash step and many use brushing. The use of an approved sanitizer along with the wash step could provide additional assurance that pathogens are not carried over into subsequent processing steps. As one producer noted, "I strongly feel that quality fruit and cleanliness are the two most important factors in good cider." Procedures such as microbiological testing and the inclusion of code numbers on labels could also be incorporated into a HACCP plan.

Overall, it appears that most Virginia cider producers are using sound production practices and are committed to producing a safe and quality product. Although some have expressed concerns regarding additional regulations and the requirement for HACCP, most have considered the use of a HACCP program. Also, most producers are interested in the use of alternative processing technologies to help ensure a safe product. Additional research in this area should be supported. As one producer commented, "We have stopped producing cider until we can evaluate final FDA regulations and alternatives to pasteurizing and risks to producing the old-fashioned cider. We must

weigh the risks, costs, and customer attitude once FDA regulations are in place. We want to utilize a cost-effective way to produce a clean product that tastes good, that customers will accept, is economical to produce, and will be competitive without high risk to us and our industry."

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Table 1. Production levels and sales location for Virginia cider producers

Annual production (thousands of gallons)					Out of state sales
<5	5-10	10-50	50-100	>100	
58.5[a]	14.75	14.75	5	7	21

[a] Values are percentages of respondents

Table 2. Orchard management practices

Practices	Percentage of respondents answering "yes"
Purchase apples from outside sources	33
Fertilize orchards with manure	8
Permit animal grazing in orchards	5
Animals present in adjacent fields	54
Use drop apples for cider	32

Table 3. Facilities

Practices	Percentage of respondents answering "yes"
Processing conducted in separate, enclosed area	79
Test water supply regularly for coliforms	72.5
Clean and sanitize equipment and facilities daily	100

Table 4. Fruit handling and processing

Practices	Percentage of respondents answering "yes"
Sort apples to remove damaged, wormy, and unacceptable fruit	98
Sort apples before washing	82
Use damaged fruit for cider	37.5
Wash apples before crushing	93
Use detergent-based fruit wash	18
Use sanitizer after washing	37
Employ brushing in conjunction with washing	64
Refrigerate apples before use	63
Prevent contamination during storage	80

Table 5. Preservation measures

Practices	Percentage of respondents answering “yes”
Pasteurize cider	22
Add preservatives	12
Chill cider immediately following production	93
Store cider refrigerated or frozen until sale	92.5

Table 6. Additional measures to help ensure safety of cider

Practices	Percentage of respondents answering “yes”
Include expiration date on label	2
Include lot or code numbers on label	14
Conduct microbiological testing on finished cider	2
Have operating HACCP program	25
Considered implementing HACCP	67
Interested in alternative processing technologies	80

SECTION IV: UTILIZATION OF ULTRAVIOLET LIGHT FOR REDUCTION OF *ESCHERICHIA COLI* O157:H7 IN APPLE CIDER

ABSTRACT

This study examined the efficacy of ultraviolet light for reducing *E. coli* O157:H7 in unpasteurized cider. Cider containing a mixture of acid resistant *E. coli* O157:H7 (6.3 log CFU/ml) was treated using a thin-film ultraviolet disinfection unit operating at 254 nm. Dosages ranged from 9,402 to 61,005 µW-sec/cm^2. Treatment significantly reduced *E. coli* O157:H7 ($p \leq 0.0001$). Mean reduction for all treated samples was 3.81 log CFU/ml. Reduction was also affected by the level of background microflora in cider. Results indicate that ultraviolet light can be effective for reducing this pathogen in cider. However, with the equipment used here, additional reduction measures are necessary to achieve the required 5 log reduction.

INTRODUCTION

Escherichia coli O157:H7 was first identified as a foodborne pathogen in 1982 and is now acknowledged as a significant cause of foodborne illness (Doyle, 1991). Illness due to *E. coli* O157:H7 can range form self-limited, watery diarrhea to the more severe hemorrhagic colitis, hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP) (Padhye and Doyle, 1992). Cattle are believed to be the primary reservoir for the organism (Borczyk et al., 1987) and the majority of foodborne outbreaks have involved contaminated ground beef (Lior, 1994).

Recent *E. coli* O157:H7 outbreaks involving contaminated deer jerky (Keene et al., 1997), mayonnaise (Erickson et al., 1995), salami (CDC, 1995), and yogurt (Morgan et al., 1993) have demonstrated the ability of this organism to survive in foods previously considered safe because of acidity, low water activity, or refrigeration. Apple cider or juice is also a novel vehicle for *E. coli* O157:H7 outbreaks and much attention has been focused on this problem.

An early outbreak of HUS associated with apple juice or cider occurred in Canada before the recognition of *E. coli* O157:H7 as a foodborne pathogen however, the responsible agent was not identified, possibly because of delays between sampling and analysis (Steele et al., 1982). Outbreaks of HUS in Massachusetts in 1991 (Besser et al., 1993) and Connecticut in 1996 (CDC, 1997) attributed to *E. coli* O157:H7 were associated with drinking contaminated apple cider. In a 1996 outbreak involving unpasteurized apple juice in the pacific northwest, Odwalla brand apple juice and juice

mixtures contaminated with *E. coli* O157:H7 were implicated and caused a nationwide recall of all products containing apple juice (CDC, 1996).

While apple juice is usually pasteurized, producers of apple cider have relied upon the products inherent acidity, refrigeration, and often chemical preservatives for preservation and protection from pathogens (Downing, 1989). However, the outbreaks mentioned above, and the results of recent research incited by those outbreaks, have generated much concern about the safety of the product as it is currently produced. Numerous studies have demonstrated the ability of *E. coli* O157:H7 to survive in cider despite its low pH and regardless of whether refrigeration or preservatives were used. Miller and Kaspar (1994) reported survival of *E. coli* O157:H7 for at least 24 hours in cider adjusted to a pH of 2. They also saw enhanced survival in cider at 4°C when compared to survival at 25°C, especially at lower pH. Survival was not affected by the use of potassium sorbate or sodium benzoate. Likewise, Zhao et al. (1993) reported longer survival times at 8°C than at 25°C regardless of whether either preservative was used.

Many believe that pasteurization is the best means of eliminating *E. coli* O157:H7 from apple cider. Some of the larger juice processors have already begun using a pasteurization procedure (Aylsworth, 1997). However, pasteurization may be cost prohibitive for many smaller operations as costs tend to increase sharply as production capacity and number of days per year of processing decrease (Kozempel et al., 1998). In addition, pasteurization may have adverse effects on the sensory characteristics

responsible for the appeal of fresh cider (Parish, 1997).

The FDA has proposed a regulation that would require fruit and vegetable juice processors to implement HACCP programs. Part of this regulation would require processors of packaged unpasteurized juice products to adapt their processes to achieve a five log reduction in the number of pathogens present in the finished product (FDA, 1997). One technology that may be a more affordable means of protection and prove to be less detrimental to ciders sensory characteristics is the use of ultraviolet (UV) radiation.

UV acts on microorganisms through its effects on nucleic acids and the greatest bactericidal effect is obtained within the range of 250-260 nm (Morgan, 1989). The sensitivity of bacteria to UV varies with species and also among different strains of the same species (Block, 1977). The dosage necessary to cause a reduction of 99.9% in *E. coli* is comparable to that required for many other vegetative bacteria (Chang et al., 1985). The possibility exists for UV inactivated bacterial cells to be reactivated by exposure to visible or near UV light in a phenomenon known as photoregeneration (Block, 1977).

This technology has been used for some time for water sterilization and has shown effectiveness against a wide variety of microorganisms (Bachmann, 1975; Yip and Konasewich, 1972). It has also been used successfully on beef (Kaess and Weidermann, 1973; Stermer et al., 1987), fish (Huang and Toledo, 1982), and poultry (Sumner et al., 1996; Wallner-Pendleton et al., 1994) to control bacteria and increase

shelf-life while causing little effect on food quality.

Little research exists on the use of UV to control pathogens in apple cider. UV has been used successfully to increase the shelf-life of refrigerated cider without affecting the flavor (USDA, 1977). Harrington and Hills (1968) reported reductions in total microbial counts in unclarified cider from an initial level of 6.6×10^5 to 1.4×10^3 CFU/ml following exposure to UV light for 54 seconds. In addition, UV treated cider showed no signs of fermentation or spoilage for up to 35 days when stored at 2.2^{o}C and the flavor was not affected.

Despite these findings, UV irradiation has not been used commercially due to the suspended solids in cider (Downing, 1989). The presence of small amounts of solutes or particulates in a liquid can greatly reduce UV penetration (Bachmann, 1975; Shama et al., 1996). Kissinger and Willits (1966) successfully eliminated microorganisms in flowing maple sap using UV radiation however, the dissolved solids content of sap is considerably lower (1-4%) than that of apple juice (9.8-16.9%) (Mattick and Moyer, 1983). For UV radiation to be effective in such liquids, the liquid must be subjected to UV in the form of a thin film (Downing, 1989). In this manner, the absorption of UV by the liquid is low and microorganisms are subjected to lethal doses (Shama, 1992). The inactivation of *E. coli* by UV light in water and humic acid solutions of high UV absorptivities through the use of a thin film apparatus was described by Shama (1992).

The goal of this investigation was to determine the efficacy of UV light for the reduction of *E. coli* O157:H7 in apple cider. The effects of flow rate during treatment

and the initial level of background microflora in cider on UV inactivation were examined.

MATERIALS AND METHODS

Test organism and culture maintenance

Five acid resistant *E. coli* O157:H7 strains 380-94, 933, C7927 (human isolate from cider outbreak), E0019, and E09 were obtained from the University of Nebraska, Lincoln culture collection. Stock cultures were maintained on Tryptone Soy agar (TSA) (Difco Laboratories, Detroit, MI) at 4° C and grown in Tryptone Soy broth (TSB) (Difco Laboratories) at 35° C. Preliminary research determined that the level of inoculum obtained in TSB for the five individual cultures was relatively even (8.0×10^8 - 1.5×10^9 CFU/ml). Each culture was subjected to two successive transfers by loop inocula to 10 ml TSB. A third transfer of 1 ml was made into 100 ml TSB acidified to pH 5 with 1 N HCl for acid adaptation (Leyer et al., 1995). Incubations were for 18-24 hours at 35° C. Cultures were then combined in equal volumes to create a five-strain mixture.

Inoculation and analysis of cider

Apple cider was obtained from local producers in Virginia and stored at 4° C or frozen until use. Frozen cider was thawed for analysis by placing at 4° C. Prior to inoculation, the level of background microflora in test cider was determined by spread plating serial dilutions (0.1% peptone) (Difco Laboratories) on Sorbitol MacConkey agar (SMAC) (Difco Laboratories) for *E. coli* O157:H7; TSA for aerobic organisms; and

Yeast and Mold agar (Difco Laboratories) supplemented with 0.01% chloramphenicol (Unipath Oxoid USA) (YMAC) for yeasts and molds. SMAC and TSA plates were incubated for 24 h at 35° C. YMAC plates were incubated for 48 hours at 25° C. In addition, the pH and sugar content (°Brix) of the test cider was determined using a pH meter (Fisher Scientific) and a Brix hydrometer (Fisher Scientific).

Three to five L portions of cider were transferred to sterile 5 L flasks and inoculated with the five-strain mixture (3.5 ml/L) to achieve an inoculum level of approximately 1.0 x 10^6 CFU *E. coli* O157:H7 per ml. Prior to UV treatment, inoculated cider was tested to determine the actual level of inoculum. Serial dilutions were made in 0.1% peptone and spread plated on SMAC and TSA supplemented with 1% pyruvic acid (Fisher Scientific) (TSAP). TSAP was used to check for sublethally injured *E. coli* O157:H7. Plates were incubated for 24 hours at 35° C. Typical sorbitol negative colonies on SMAC and typical colonies on TSAP were enumerated with a Quebec colony counter. Questionable colonies present on TSAP were verified using biochemical and serological tests. Routine verification of isolates was conducted using Micro-ID's (Remel, Lenexa, KS), and *E. coli* O157 Latex agglutination test kit (Unipath Oxoid USA).

UV treatment

Inoculated cider was pumped through a thin film UV disinfection unit , model CIDER-10uv (Ideal Horizons, Poultney, VT) using an air operated diaphragm pump (Graco, Inc., Minneapolis, MN) or a peristaltic pump (Cole-Parmer Instrument Co.,

Vernon Hills, IL) depending on the desired flow rate. The UV disinfection unit operates at a peak radiation of 254 nm with a maximum intensity of approximately 107,366 $\mu W/cm^2$. Because fouling of the quartz sleeves which enclose the lamps tends to increase and the efficiency of the lamps themselves tends to decrease over time, the applied intensity is somewhat less than maximum. The intensity of the unit as rated by the manufacturer is actually the end of lamp life intensity which is 64,420 $\mu W/cm^2$ or 60% of the theoretical maximum. This is also the figure used in this research. The unit incorporates ten individual UV chambers that are connected in series. UV dosage was varied by adjusting the flow rate of cider through the UV disinfection unit.

Cider flow rates (GPM) were measured with a flow meter (Fisher & Porter Co., Warminster, PA) attached to the outlet tubing on the UV disinfection unit. Following UV treatment, cider was collected in a sterile 2 L flask. Recovered samples were immediately plated and analyzed as detailed above for inoculated cider.

Prior to and following each treatment session, the UV unit and tubing were cleaned, sanitized, and rinsed. Cleaning and rinsing were accomplished by pumping hot water through the unit. A 500 ppm hypochlorite solution was used for sanitizing. Following the rinse step, the UV lamps were turned on for ten minutes. Holding and collection vessels for cider were cleaned and autoclaved before use.

Experimental design and statistical analysis

The experiment was run a total of 25 times at flow rates ranging from 0.264 GPM to 1.713 GPM. This corresponds to minimum and maximum UV dosages of 9,402 and

61,005 µW-sec/cm^2, respectively. Microbial counts (CFU/ml) were determined in duplicate for each replication. Counts were converted to log values and differences between untreated and UV treated cider for each replication were calculated as log reduction factors (LRF; log CFU/ml). Data were analyzed by regression analysis using the Statistical Analysis System (SAS Institute, Cary, NC) to determine significant differences among treatments.

RESULTS & DISCUSSION

E. coli O157:H7 was not detected in any of the test ciders prior to inoculation. Background populations of aerobic mesophilic bacteria in test ciders ranged from undetectable (<1 log CFU/ml) to 4.40 log CFU/ml with a mean of 2.18 log CFU/ml. Background populations of yeasts and molds ranged from undetectable to 4.73 log CFU/ml with a mean value of 3.39 log CFU/ml. The pH and sugar content did not vary greatly among the ciders used for testing. The pH of uninoculated test ciders ranged from 3.62 to 3.85 with a mean of 3.74. Sugar content ranged from 12.0 to 12.3 with a mean of 12.2^o brix.

Populations of *E. coli* O157:H7 in inoculated cider prior to UV treatment as enumerated on SMAC ranged from 5.32 to 6.49 log CFU/ml with a mean value of 6.01 log CFU/ml. Using TSAP for the recovery medium, populations ranged from 5.72 to 6.75 log CFU/ml with a mean of 6.29 log CFU/ml. The somewhat higher recovery on TSAP suggests that a portion of the cells were injured, possibly due to the pH of the

cider or the presence of organic acids.

UV treatment significantly reduced *E. coli* O157:H7 in apple cider ($p \leq 0.0001$). The mean LRF for all UV treated samples was 3.81 and 3.64 log CFU/ml for SMAC and TSAP respectively. This corresponds to a kill rate of >99.9%. In comparison, Harrington and Hills (1968) reported an 88% reduction in total microbial counts for cider pumped through a UV irradiation unit at a rate of 0.383 GPM.

The lower reduction for TSAP may indicate apparent sublethal injury, possibly due to exposure to UV. However, the difference in reduction between SMAC and TSAP was not statistically significant. In addition, since injured cells were recovered both before and after UV treatment, not all injury can be attributed solely to UV treatment. Since the results did not differ significantly between media, only the results from SMAC will be presented.

The relationship between UV dosage, the pre-treatment level of background yeasts and molds in cider, and reduction of *E. coli* O157:H7 is shown in Table 1. Due to difficulties in reproducing flow rates during replicate treatments, the values reported are means for samples processed within a range of 2,000 μW-sec/cm^2. The UV dosages reported are averages of all dosages within a particular range. Because yeast and mold levels are typically higher in unpasteurized cider (Downing, 1989), data for samples with background yeast and mold populations of <2.0 log CFU/ml are not included in Table 1 with the following exception. Both samples processed at the highest UV dosage had yeast and mold levels of 1.0 log CFU/ml or less and data for these samples is included

for comparison. Yeast and mold levels are used here instead of aerobic bacteria because yeast and mold populations were generally higher than that of aerobic mesophilic bacteria. In addition, yeast cells are larger than bacteria and thus, require lower numbers to cause turbidity (Kissinger and Willits, 1966).

Since the level of background microflora was difficult to control, i.e. samples tested later generally had higher levels, it was impossible to process samples with the same level at all UV dosages. However, the mean level of yeasts and molds varies by less than 1 log CFU/ml except for the top row in Table 1. As expected, the best reduction resulted when samples with very low levels of background microflora were processed at the highest UV dosage.

In general, the LRF increases with an increase in UV dosage. This relationship is depicted graphically in Figure 1. However, the effect of UV dosage on reduction was not statistically significant. In addition, the level of background yeasts and molds in cider appears to have some effect. In fact, regression analysis found that the pre-treatment levels of yeasts and molds in cider significantly affected reduction ($p \leq 0.03$). Although not shown in Table 1, LRF's of 5 log CFU/ml or greater were only obtained when the combined level of background microflora was approximately 3 log CFU/ml or less, regardless of UV dosage.

Background microbial populations in liquids along with particulates and organic matter have been associated with low transmissivity of UV light (Shama et al., 1996). These findings are also consistent with those of Kissinger and Willits (1966) who

reported that UV reduction of microorganisms in maple sap was less effective in the presence of high levels of yeast.

Figure 2 illustrates the relationship between LRF's for UV treated cider and pre-treatment levels of background yeasts and molds through linear regression analysis. The correlation coefficient was 0.44 suggesting that at least some of the variation in LRF's may be due to variances in yeast and mold levels. Higher levels of yeasts and molds in cider prior to treatment corresponded with lower LRF's.

As mentioned previously, the best reduction was obtained when cider with very low initial levels of yeasts and molds (1 log CFU/ml) was subjected to the highest UV dosage tested (61,005 µW-sec/cm^2). *E. coli* O157:H7 was reduced by an average of 5.4 log CFU/ml in such instances. This would meet the FDA requirement for a five log reduction. However, levels of background microflora, especially yeasts, in cider are typically much greater than this (Downing, 1989). In addition, with the disinfection unit used for this research, a dosage this high was only achieved when cider was processed at a flow rate of approximately one L per minute. A flow rate this slow is probably impractical for use in a commercial setting.

The mean level of *E. coli* O157:H7 in cider prior to UV treatment of approximately 6 log CFU/ml would appear to represent extreme levels of contamination. However, this level of contamination was necessary to conduct these studies. In comparison, the mean coliform count in 59 cider samples from 12 different producers was 2.74 log CFU/ml (Silk et al., 1997). For the same samples, the mean level of

heterotrophic bacteria was 4.64. The highest coliform counts were for samples which had been produced using dropped apples (Silk et al., 1997). In another study involving samples from 15 facilities, the average aciduric population of unpasteurized juice, including aciduric yeasts and molds, was 5 log CFU/ml (Swanson et al., 1985).

With the equipment used in this study, the FDA requirement for a 5 log reduction of *E. coli* O157:H7 in unpasteurized cider would not likely be met using UV light alone. However, it could be met by using a combination of methods, including UV. Achieving such a reduction solely through the use of UV light would only be possible for cider with very low levels of background microflora processed at extremely slow rates. However, modifications to the unit to increase the intensity of UV irradiation as well as the maximal flow rate may make it possible to achieve greater reduction at faster processing speeds. Reduction will vary with the equipment used for UV disinfection as well as the nature of the liquid being treated. For this reason, it would be wise to investigate the effectiveness of UV for use in a particular situation (Bachmann, 1975).

Some advantages to the use of UV for the treatment of foods include the lack of residual chemicals and radiation, no changes in physical characteristics, and low capital and maintenance costs (Morgan, 1989). The results of this investigation suggest that the potential exists for the use of UV light to help assure the safety of unpasteurized apple cider. At the very least, UV treatment could be used in conjunction with other preventive measures such as GMP's and sanitizing treatments for apples, as part of an approved HACCP plan. In other research conducted in this laboratory, treatment of

apples with 5% acetic acid resulted in a mean *E. coli* O157:H7 reduction of 3.1 log CFU/cm^2 (Wright et al., 1999). According to the FDA (1997), processors of unpasteurized juices would be free to employ a combination of methods to achieve the required 5 log reduction.

More research is needed to identify the actual UV dosage necessary to achieve a 5 log reduction of *E. coli* O157:H7 in unpasteurized apple cider containing typical levels of background microflora. In addition, the effect of UV treatment on the sensory characteristics of cider should be addressed. Also, since the effectiveness of UV reduction of *E. coli* O157:H7 appears to be affected by background microbial populations in cider, this matter should be considered when conducting future studies. Finally, once an optimal dosage has been identified, researchers should consider the phenomenon of photo-regeneration in UV treated cider.

ACKNOWLEDGMENTS

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Table 1. The effect of UV dosage on the reduction of *E. coli* O157:H7 in apple cider containing the following pre-treatment levels of yeasts and molds.

Average UV Dose[a] (μW-sec/cm^2)	Log Reduction Factor[b] (log CFU/ml)	Background Yeasts & Molds (log CFU/ml)
61,005 (n=2)	5.40[c]	1.00[c]
29,076 (n=3)	3.58	4.70
18,641 (n=1)	3.02	4.73
14,713 (n=4)	3.01	4.17
12,100 (n=2)	2.89	3.85
10,288 (n=7)	3.09	3.98

[a] Average UV dosage for all samples processed within a range of <2,000 μW-sec/cm^2

[b] Difference in microbial counts on Sorbitol MacConkey agar between untreated and UV treated cider

[c] Values are means for all samples processed within each UV dosage range

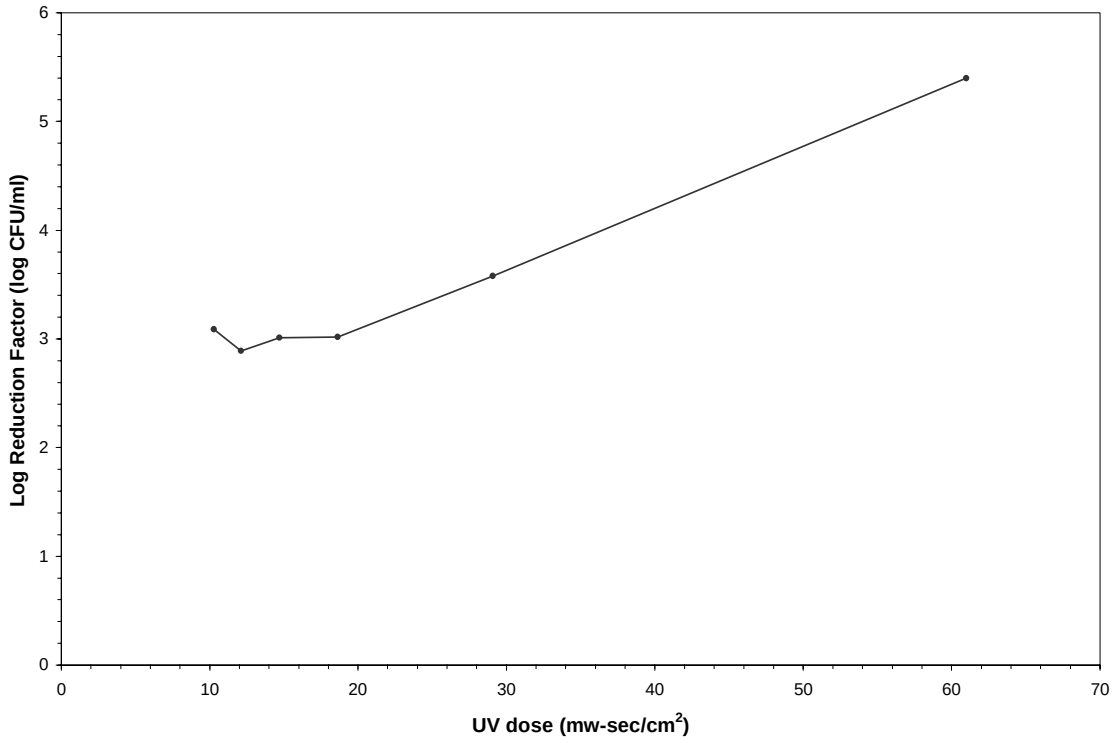

Figure 1. The effect of UV dosage at 254 nm on reduction of *E. coli* O157:H7 in apple cider.

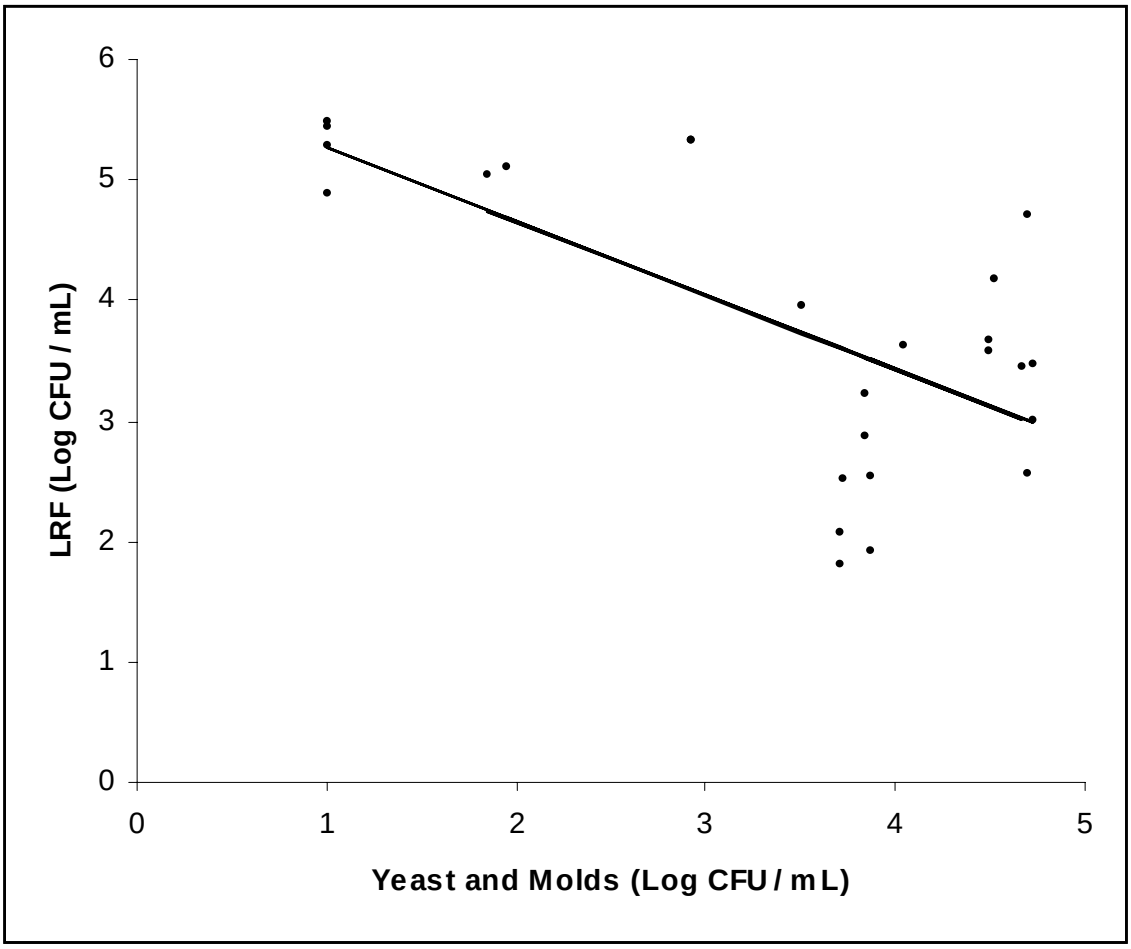

Figure 2. Linear regression analysis of *E. coli* O157:H7 in apple cider treated with UV light; background yeast and mold population vs. Log Reduction Factor (LRF)[a] (r^2=0.44).

[a] Difference in microbial counts on Sorbitol MacConkey agar between untreated and UV treated cider

APPENDICES

APPENDIX A: Survey of Virginia cider producers practices

June 23, 1998

Dear Virginia Cider Producer:

We are currently conducting research, which is aimed at identifying alternatives to pasteurization to help assure the safety of apple cider. To best apply the information gained from this research to the cider industry in Virginia, it is necessary to gauge current practices. This survey will enable us to have a better understanding of current production practices and help to identify areas where improvements are possible. Your help would be greatly appreciated. Please take a few minutes to complete the survey and return it in the enclosed, postage paid envelope by August 1, 1998.
Please do not include the names of any individuals or your company name on the survey or the envelope. This information is not needed. The answers to the survey questions will simply be tallied to establish percentages for the industry as a whole. Again, there is no need to identify yourself or your company.
Thank you for your assistance.

Jim Wright
Graduate Research Assistant

Dr. Susan Sumner
Associate Professor

VIRGINIA CIDER PRODUCERS SURVEY

1. How many gallons of cider does your facility produce in a typical year?

____ <5,000 ____ 5,000 – 10,000 ____ 10,000 – 50,000

____ 50,000 – 100,000 ____ >100,000

2. Do you ship cider for sale out of state?

____ yes ____ no

3. Do you

____ grow all your own apples ____ purchase some apples from outside sources

____ purchase all apples from outside sources?

4. Do you use manure to fertilize orchards?

____ yes ____ no

5. Are domesticated animals allowed to graze in the orchards where your apples are obtained ?

____ yes ____ no

6. Do domesticated animals graze in fields adjacent to apple orchards?

____ yes ____ no

7. Do you use drop apples for cider production?

____ yes ____ no

8. If so, approximately how often are drop apples gathered?

____ daily ____ twice per week ____ weekly

____ biweekly ____ monthly ____other

9. Is all processing carried out in a separate, enclosed room or building?

____ yes ____ no

10. Are apples sorted to remove damaged, wormy, or unacceptable fruit?

____ yes ____ no

11. If so, are apples sorted prior to washing/brushing?

____ yes ____ no

12. Is damaged fruit used for cider production?

____ yes ____ no

13. Are apples washed prior to crushing?

____ yes ____ no

14. If so, do you use any type of detergent-based fruit wash?

____ yes ____ no

15. Do you use a sanitizer such as a chlorine solution after the initial wash step?

____ yes ____ no

16. Is your water supply regularly tested for coliform bacteria?

____ yes ____ no

17. Is brushing employed along with washing?

____ yes ____ no

18. Are apples stored between 32° and 40° F prior to pressing?

____ yes ____no

19. Are apples stored in such a way as to prevent contamination from rodents, birds, or insects?

____ yes ____ no

20. Do you clean and sanitize all processing equipment and facilities after each day's operation?

____ yes ____ no

21. Do you pasteurize your cider?

____ yes ____ no

22. Indicate if you use any of the following preservatives in your cider.

____ potassium sorbate ____ sodium benzoate

23. Is freshly pressed cider chilled immediately following production?

____ yes ____ no

24. Is bottled cider stored between 32° and 40° F or frozen until sale?

____ yes ____ no

25. Are cider containers labeled with an expiration date?

____ yes ____ no

26. Are cider containers labeled with an identifying lot or code number?

____ yes ____ no

27. Is microbiological testing performed on cider prior to sale?

____ yes ____ no

28. Do you currently have an operating HACCP program?

____ yes ____ no

29. If not, have you considered implementing a HACCP program at your facility?

____ yes ____ no

30. Would you be interested in using alternative processing technologies, if available, to help assure the safety of your cider?

____ yes ____no

Please include any additional comments below. If you need more space, use the back of this page.

Thank you for taking the time to complete this survey. Your response is greatly appreciated. If you have any questions, please contact Dr. Susan Sumner at 540-231-5280 or Jim Wright at 540-231-8845.

APPENDIX B: VIRGINIA CIDER PRODUCERS SURVEY

COMMENTS FROM PRODUCERS

- "Most of our apple trees are wild so cider production is the only suitable way to market these apples. If laws become more stringent, I will cease production."
- "This is a small, one man operation producing 250-500 gal per year. I am interested in learning about new developments in low cost juice purification. If UV equipment similar to the UV water purifier I have in my home becomes available, I would like to know the details of cost, installation, etc."
- "I strongly feel that quality fruit and cleanliness are the two most important factors in good cider."
- "Even with all this (research into alternative processing technologies?), we have decided it is best that we do not produce any more cider for sale."
- "I feel unpasteurized cider is safe when clean and careful producing practices are used. I will quit before bowing to unnecessary regulations! I feel that the media and the powers that be have been unfair and unrealistic in giving unpasteurized cider a bad rap. To my knowledge, only one person, a 12 year old in Colorado (and that is bad) has died from unpasteurized cider. What was her health condition otherwise? Instead of outlawing unpasteurized cider, why not regulate it with periodic, unannounced visits to secure samples and weed out those producers that do not use safe cider producing practices. We are regulating ourselves to death! It is a wonder any of us are alive since I don't see how our

parents lived long enough to have children without all these regulations. Perhaps we need to enact a law not to pass additional laws that are not really necessary. Regulate by periodic testing but don't outlaw a practice that is centuries old and is preferred by most people that know what good unpasteurized cider is all about."

- "This is a small, <30 gal per week, mom and pop outfit. Culls from a fresh roadside stand operation are pressed for fresh cider which is usually sold the day of pressing, often to those who helped press it. Don't forget the little guy when dreaming up regulations or requirements."
- "Cider making is a process to help apple producers to stay in the apple business."
- "We are a small operation and desire to continue producing raw cider. We feel SOP (Standard Operating Procedures) has been adequate for us because we do have control over what we do and use for cider production. The only procedure we would be able to afford would have to be very inexpensive as we could not produce cider (ie. Ultraviolet). Thank you for your research."
- "We do not press or make our cider but get it custom made with our apples. The owner (of the mill that produces our cider) is real discouraged and feels he will shut down if (regulations) require an additional major expense to his operation. This is not his major source of income, more of a hobby."
- "At present, unless there is a mandate from USDA to pasteurize cider this season, I do not plan to do so. I plan to continue as I have done in the past. We have not

had any instances of contamination."

- "I do not understand how good fruit picked off of a tree and mashed and pressed with (clean?) equipment and kept cool can be bad!"
- "The risks do not justify the overkill of the new HACCP rules!"
- "We have stopped producing cider until we can evaluate final FDA regulations and alternatives to pasteurizing and risks to producing the old fashioned cider. We must weigh the risks, costs, and customer attitude once FDA regulations are in place. We want to utilize a cost effective way to produce a clean product that tastes good, that customers will accept, is economical to produce, and will be competitive without high risk to us and our industry. I'm not confident that this is in the cards. Anyone want to buy a new cider operation at a low price?? Please send results of survey to all persons who receive this survey."

VITAE

Jim Wright was born in Kane, Pennsylvania and he spent most of his early years in Indiana County, Pennsylvania and graduated from Homer-Center High School. He also attended Indiana University of Pennsylvania and received his Bachelor's degree in Biology in May of 1992. At IUP, Jim worked as a laboratory assistant in the Biology department.

Following graduation, Jim accepted a position with the United States Department of Commerce/National Marine Fisheries Service. He served as a microbiologist for four years in USDC's National Seafood Inspection Laboratory located in Pascagoula, Mississippi. It was during this time that he became interested in food science and decided to further his education in this field.

He enrolled in the Master's program in Food Science and Technology at Virginia Polytechnic Institute and State University in January of 1997. While at Virginia Tech, he was a member of the Institute of Food Technologists; the International Association of Milk, Food, and Environmental Sanitarians; and Gamma Sigma Delta.